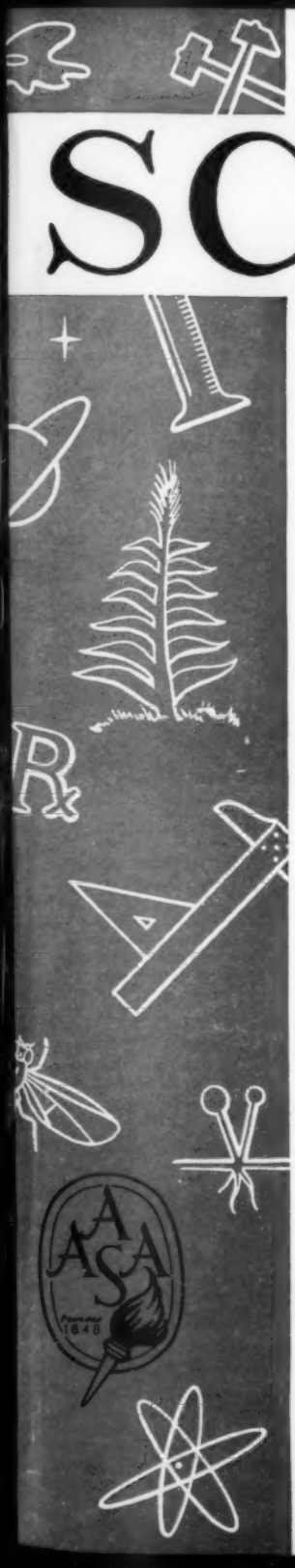

SCIENCE

SEPTEMBER 12, 1952

VOLUME 116 NUMBER 3011

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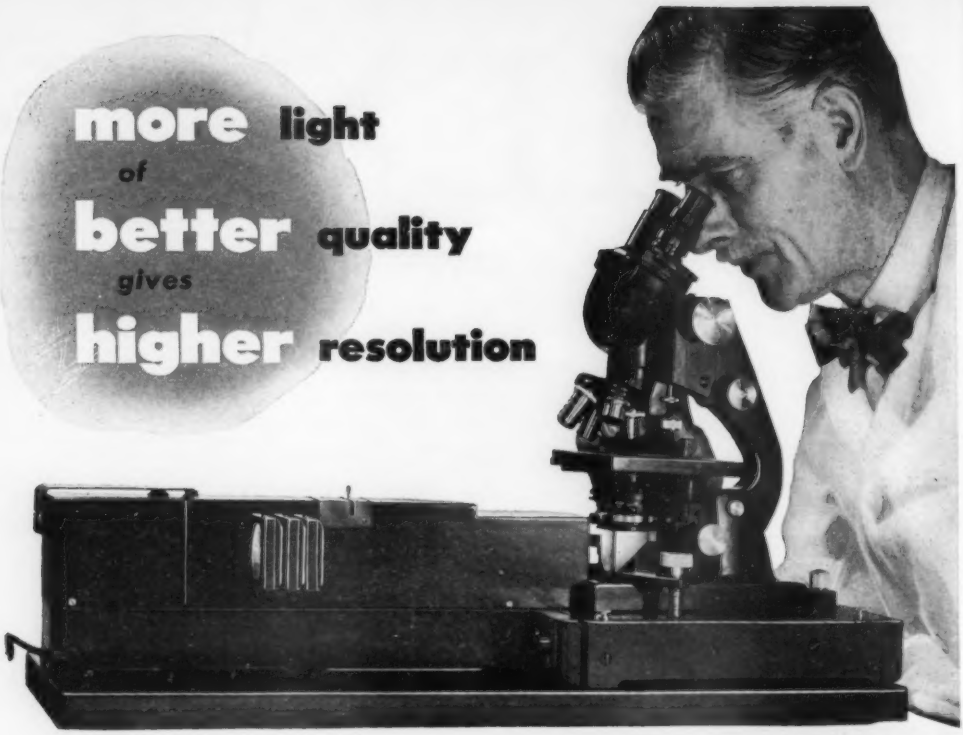

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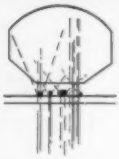

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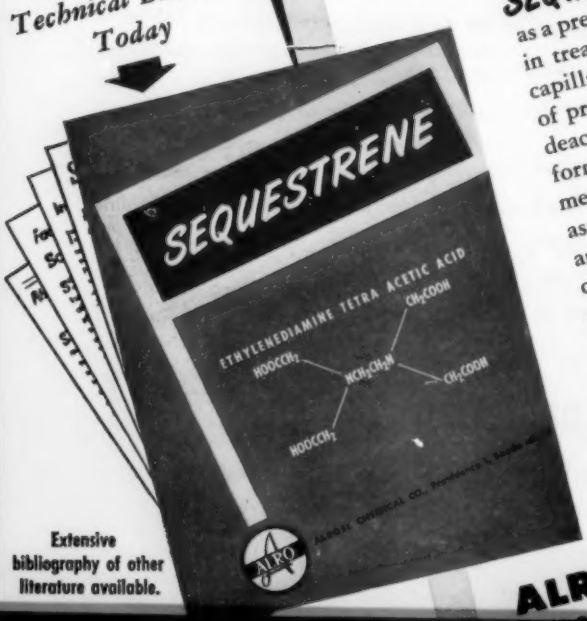

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AAAS Grants and Prizes

In 1951 the AAAS awarded 46 grants-in-aid for research, using the income from its modest endowment funds, as well as special contributions received for this purpose. The amounts given each recipient were relatively small but, with the exception of two grants made by the Ohio Academy of Science to the Committee on Ohio Flora, they were given in support of individual projects. All but one of them were administered by affiliated academies of science, to each of which the AAAS remits $50 or a sum computed at fifty cents per academy member who is also a member of the Association, whichever amount is greater. Most of the academies have taken advantage of this arrangement, and a few of them match the amount made available from Association funds.

For several years endowment funds have yielded somewhat higher earnings, and the Association is now in a position to offer annually a new grant of $500. This year the money will be drawn from the income of the A. G. Stillhamer Endowment Fund and will be awarded for a research project in the field of biology. A special research award for 1952, not to exceed $500, will also be made, and it is hoped that the names of the recipients can be announced at the St. Louis meeting in December. Additional details regarding these two research grants appear elsewhere in this issue, together with an announcement of the $1000 Prize in the Social Sciences.

The lengthening list of prizes and grants administered by the Association has prompted a review of the relative merits of prizes, which are awarded for work already done and adjudged good, and of grants-in-aid, which are given on the basis of promise that significant work will be competently performed. At the present time the policy pursued by the Association is fortuitous, and in large part it reflects the wishes of the donors who have asked the Association to administer gifts or bequests. The current thinking of the Executive Committee favors the grant-in-aid, if only because the applicant, if adequately supported, may produce not merely something new, but something of the utmost significance in one of the fields of science. It should be noted that many of the recipients of prizes have turned the prize money back into research; few of them have regarded it simply as a windfall for which no further professional obligation was incurred. In retrospect there seems to be equal justification for the award of funds either to encourage research or to recognize it after it is done.

This year the awards that have been made, or that will be made as a feature of the 119th Meeting of the AAAS at St. Louis, are sufficient in number to warrant listing, in order that readers of SCIENCE may know about this phase of the Association's activity and interest. Complete lists of past recipients will appear in the General Program and Directory.

AAAS Academy Research Grants (administered by 38 affiliated state academies of science)	$7000
Special Academy Grants for Alabama and British Columbia (for genetics and eugenics)	400
Stillhamer Research Grant in Biology	500
AAAS Research Grant	not to exceed 500
Newcomb Cleveland Prize (for a paper of recognized significance presented before a scientific session at the annual meeting)	1000
Theobald Smith Award (provided by Eli Lilly and Company for a distinguished contribution to medical science by a person under 35 years of age)	1000
AAAS–George Westinghouse Newspaper Science Writing Award	1000
AAAS–George Westinghouse Magazine Science Writing Award	1000
AAAS Thousand Dollar Prize in Sociology (for sociological theory employing successfully methods used in the natural sciences)	$1000

HOWARD A. MEYERHOFF
Administrative Secretary, AAAS

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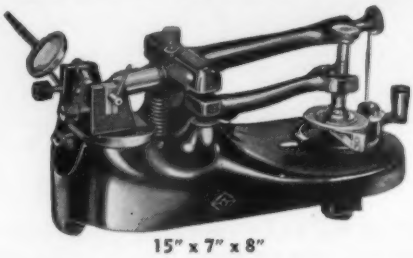

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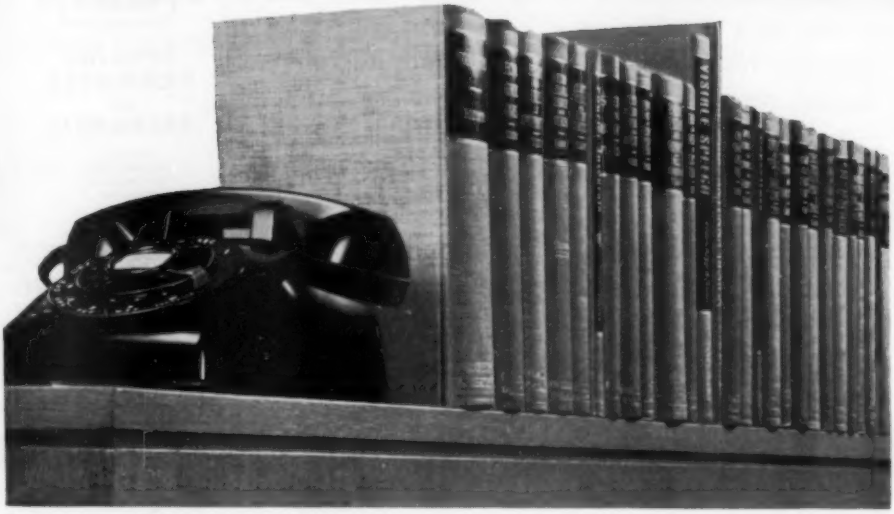

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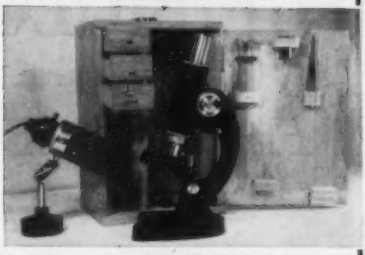

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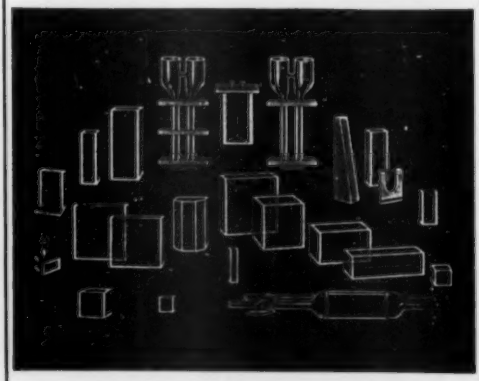

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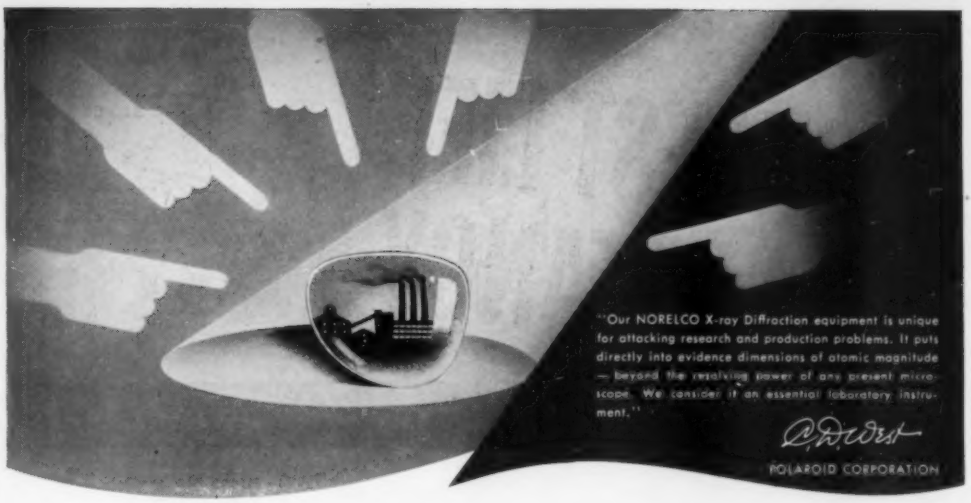
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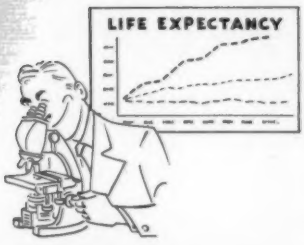

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Nucleic Acid Content of the Squamous Cancer Cell

Robert C. Mellors,[1] John F. Keane, Jr., and G. N. Papanicolaou

Sloan-Kettering Institute and *Pathology Laboratories, Memorial Center for Cancer and Allied Diseases,* and the *Departments of Pathology and Anatomy, Cornell University Medical College, New York*

AS A RESULT of the chemical analysis and the particle count of nuclear suspensions derived from the tissues of several mammalian animals, it has been shown (*1, 2*) that the quantity of desoxypentose nucleic acid (DNA) of the nucleus is constant for the interphase somatic (diploid) cells of a particular species, that the amount in diploid nuclei is very similar for different species of mammals, and that this quantity is twice that of haploid nuclei. This concept of the relative constancy of the DNA of the interphase nucleus has been extended to other classes of animals (*3*).

Although exceptions have been noted (*4*), microphotometric methods for the determination of the relative DNA in Feulgen-stained nuclei in the tissues of higher animals confirm this concept (*5–10*) and indicate that some tissues, notably the liver and the pancreas, contain nuclei with one, two, and four times the diploid amount of DNA (*11*). High order polyploidy and correlating DNA values are observed in lower animals (*11*); studies on the cells of plants show either inconstancy (*12*) or constancy (*13*) of the DNA per nucleus; and under other conditions, the DNA per nucleus is observed to be altered spontaneously (*14–16*) or experimentally (*14, 17–19*). A permissible appraisal of the published data is that, although there is often a certain average and modal quantity of DNA per nucleus in a particular type of cell, there is a frequent occurrence of values that are greater and lesser than this modal quantity. Such a dispersion of values may be due to actual variations in the composition of the nucleus and to experimental errors in the methods of measurement.

A number of investigations have dealt with the relative content of DNA and on properties related thereto in normal and neoplastic cells (*5, 6, 20–35*). The DNA content per nucleus as determined chemically is about twice as great in the Ehrlich ascites tumor of mice as in normal somatic cells of this animal (*24, 25, 28*), and this finding is confirmed by the microphotometric analysis of individual cells (*6*). Among the pertinent cytological observations on clinical material are the increases in the nuclear volume (*36*), the chromosome volume (*37–39*), the ultraviolet absorption caused by nucleic acids (*20*), the Feulgen-stained DNA (*34–35*), and the affinity for basic dyestuffs of certain types of cancer cells.

The purpose of the present paper is to determine by microabsorption spectroscopy the quantity of nucleic acid in nuclei of epithelial cells of the mucosa of the human cervix uteri and of squamous cancer cells originating in this tissue. The total quantity of nucleic acid is estimated so that the data pertain to the summation of DNA and of ribose nucleic acid (RNA) and not to a particular type of nucleic acid.

Methods

Cytological material. This consists of cells that are obtained from the pars vaginalis of the cervix uteri by the use of a cotton applicator moistened with physiological saline solution or by means of an aspirating pipette. The applicator is rolled onto slides of optically polished quartz (Corning Vycor No. 791) so as to distribute the cells without compression and distortion. The slides are immediately placed in anhydrous glycerin, in about one third of the cases, or in 10 per cent formalin-saline; all are promptly mounted in glycerin with a quartz cover slip and a paraffin seal and are then available for the selection of cells for microscopic analysis.

Duplicate specimens are taken for staining by the method of Papanicolaou (*40*). The stained preparations are studied, and, with this background of experience and by means of a phase microscope, representative and nondegenerated epithelial cells are selected in the unstained slides and are classified by the morphological criteria of Papanicolaou (*41*) as follows: Class I: negative; epithelial cells, such as squamous and parabasal cells, with no atypical or abnormal features; Class II: negative; atypia, but no abnormal features; Class III: abnormal features, suggestive of, but not conclusive for, cancer cells; Class IV: fairly conclusive for cancer cells; Class V: conclusive for cancer cells.

The material on which this report is based was obtained from twenty patients of whom, as shown by biopsy, eleven are negative for uterine cancer, seven have squamous or epidermoid carcinoma of the cervix, and two have intra-epithelial squamous carcinoma of the cervix. Cells of Class I and Class II were obtained

[1] This work was done under a Damon Runyon Senior Clinical Research Fellowship of the American Cancer Society and was supported in part by a research grant from the National Cancer Institute, National Institutes of Health, USPHS.

from the noncancerous group, and those of III, IV, and V from the cancerous group.

Microspectrography. The analytical instrument, referred to elsewhere as system II (*42–43*), consists of an ultraviolet light source, reflecting microscope (numerical aperture, 0.56), and quartz spectrograph. The microscope condenser focuses the continuous spectrum (wavelength, 0.2–0.4 μ) of the source on the object, such as a cell and the adjacent mounting medium, and the reflecting objective images the object on the entrance slit of a spectrograph (*42, 44*). The images are dispersed in the spectrograph and refocused on a photographic plate (Kodak 103-0 UV or Tri X Pan, type B) as a series of overlapping monochromatic images, referred to as a *microspectrogram*. The objective magnification is 50; thus, with an entrance slit 100 μ in width, the light admitted to the spectrograph may be considered as passing through successive square areas of the object that measure 2 μ by 2 μ or 4 μ². By the nature of the microspectrographic procedure, the light transmission is integrated over this object area, the image of which becomes essentially homogeneous.

Standard methods of photographic photometry are used (*45*): plate calibration is carried out by means of a rotating sector, and photographic developing conditions are rigorously controlled. A recording densitometer with an illuminating aperture 50 μ in diameter is used to scan the microspectrogram in one direction in order to relate the absorption with wavelength, and in the other direction to determine the absorption in various morphological components. In this investigation, more than 1000 plates were utilized, and a minimum of 24 microspectrograms were recorded on each cell, with varying conditions of time of exposure, width of slit, and focus. A quantitative analysis of about one third of the accumulated spectra is reported herewith.

Calculations. A graphic computation is made of the extinction $\left(E\lambda = \log_{10}\frac{I_0}{I}\right)$ at various wavelengths of light from 240 to 350 mμ where I_0 is the incident intensity, and I is the transmitted intensity of light with wavelength λ. In nuclear material a broad absorption band occurs in the region 250–280 mμ with an extinction maximum, designated E_{260}, in the region of 260 ± 5 mμ.

The quantity (Q) of nucleic acid per nucleus is given by the expression:

$$Q = C \cdot V,$$

where C is the concentration of nucleic acid, and V is the volume of the nucleus expressed in appropriate units.

The concentration, as given by the Bouguer-Lambert-Beer law, for the absorption of radiation by a solute molecularly dispersed in a nonabsorbing solvent is:

$$C = \frac{E_{260}}{\varepsilon_s l}$$

where C is in g/l, l is the optical path length in cm, and ε_s is the specific extinction coefficient, which for nucleic acid is 20 (the extinction at 260 mμ for a concentration of 1 g/l and an optical path of 1 cm).

The volume of the nucleus when treated as a flattened (oblate) spheroid approaches that of an enclosing elliptical cylinder with surface area $A = \pi ab$, and thickness l, so that

$$V = A \cdot l = \pi ab \cdot l,$$

where a and b are the semimajor and the semiminor axes, respectively, of the nuclear surface, and l is the thickness.

For an ellipsoid, the volume is two thirds that of the enclosing elliptical cylinder, and

$$V = \frac{2}{3} A \cdot l = \frac{2}{3} \pi ab \cdot l.$$

The quantity of nucleic acid per nucleus, for the case of the elliptical cylinder, becomes

$$Q = \frac{E_{260}}{20 \cdot l} \cdot A \cdot l \cdot 10^{-6} = \frac{E_{260} \cdot A}{20} \cdot 10^{-6}\ \text{g}.$$

where A is in cm² and Q is in g. If A is expressed in μ², the equation becomes

$$Q = \frac{E_{260} \cdot A'}{2} \cdot 10^{-12}\ \text{g} = \frac{E_{260} \cdot \pi a'b'}{2} \cdot 10^{-12}\ \text{g},$$

where a' and b' are the nuclear semiaxes in μ, which may be accurately measured from phase photomicrographs (500–1000 times enlargements) of the cell nuclei. The computations of Q values in this paper are made on the basis of both an elliptical cylinder and an ellipsoid, the values in the latter being two thirds that for the cylinder. The uncertainty in the computation resides in the fact that at the time this work was undertaken, there was no method for the exact determination of the three-dimensional spatial configuration of the nuclear volume. Recent observations in our laboratory by means of multiple-beam interference microscopy indicate within an accuracy of 0.02 μ that the optical paths (product of thickness and refractive index) in isolated, optically homogeneous nuclei are those to be derived from a volume that is intermediate between an ellipsoid and an elliptical cylinder.

Results

Nuclear extinction. The average values of the extinction at 260 mμ, E_{260}, are given in Table 1 for the nuclei of epithelial cells of the cervix uteri. As previously stated, the extinction as determined from a microspectrogram is the integrated value for a central 4 μ² area of the nucleus. Cells of Class I and Class II combined have extinctions of the order of 0.24 and 0.33, whereas those of Class III and of IV and V combined have values of 0.51 and 0.54, respectively. The differences between the negative Class I and Class II combined and the abnormal Class III, or between the negative classes and the cancer classes, IV and V, combined, are statistically significant and have, by chance alone, a probability of occurrence of the order of one in a few thousand to one in several thousands.

TABLE 1

NUCLEAR EXTINCTION AT 260 mμ*
(Epithelial Cells of the Cervix Uteri)

Cell class or type	No. cells	E_{260}	SD	Comparison			
				Classes	Δ	SE Δ	$\frac{\Delta}{SE\,\Delta}$
I and II (Sq)	23	.24	.10	Sq and Pb	.09	.04	2.2
I and II (Pb)	21	.33	.15	(Sq and Pb) and (III and IV and V)	.25	.04	6.2
III	20	.51	.22	III and Pb	.18	.05	3.5
IV and V	31	.54	.23	(IV and V) and Pb	.21	.05	4.0

* Average nuclear extinction at 260 mμ, E_{260}; standard deviation, SD, of average; difference, Δ, between classes; standard error, SEΔ, of this difference; and ratio, $\frac{\Delta}{SE\Delta}$, which for values greater than 3 indicates an extremely remote probability of occurrence of the difference by chance alone. Symbols: Sq, squamous; Pb, parabasal; I–V, classification of Papanicolaou.

Nuclear dimensions. The nuclear semiaxes (the largest and the smallest), the nuclear areas computed as ellipses, and the volumes calculated as ellipsoids are given in Table 2 for a random sample of epithelial cells of the cervix uteri. The average area of the nucleus is 62 μ^2 for I and II combined and 112 μ^2 for Class III and IV and V combined, and the average volumes are 335 μ^3 and 760 μ^3, respectively; that is, the nuclear volume of the category of abnormal and cancer cells is approximately twice that of the class of normal and atypical cells.

Nucleic acid content. The nucleic acid content per nucleus in resting (interphase) epithelial cells of the cervix uteri is given in Table 3. The values have been computed from the extinction at 260 mμ as measured for individual nuclei and for two representations of

TABLE 2

NUCLEAR SEMIAXES, AREAS, AND VOLUMES*
(Epithelial Cells of the Cervix Uteri)

Cell class or type	No. cells	Semimajor		Semiminor		Area	Vol
		$a(\mu)$	SD	$b(\mu)$	SD	$\pi ab\,(\mu^2)$	$\frac{4}{3}\pi ab^2(\mu^3)$
I and II (Sq)	58	4.8	1.3	3.8	0.8	57	290
I and II (Pb)	52	5.1	1.0	4.3	0.7	69	395
I and II (total)	110	4.9	1.2	4.0	1.4	62	335
III	27	6.7	1.9	5.0	1.1	105	698
IV and V	27	7.2	1.8	5.3	1.7	120	845
III and IV and V	54	7.0	1.9	5.1	1.5	112	760

* Average nuclear semimajor axis, a, and semiminor axis, b, in μ; standard deviation, SD, of these averages; average nuclear area, in μ^2 and volume (as an ellipsoid) in μ^3. Symbols as in Table 1.

TABLE 3

NUCLEIC ACID CONTENT PER NUCLEUS*
(Epithelial Cells of the Cervix Uteri)

Cell class	No. cells	Nucleic acid per nucleus, 10^{-12} g					
		Determined by $Q=\frac{E_{260}\cdot A}{2}$			Theoretical, $Q=K2^n$		
		Elliptical cylinder		Ellipsoid			
		Av	SD	Av	$K=4.5$	$K=3$	n
I and II	24	9.3	5.0	6.2	9	6	1
III, and IV and V	34	35		23			
Subgroup 1	17	17	4.4	11	18	12	2
" 2	8	38	2.5	25	36	24	3
" 3	9	67	9.8	45	72	48	4

* Average nucleic acid content per nucleus determined by microspectroscopy of individual nuclei and computed for a nuclear volume that is an elliptical cylinder and an ellipsoid; and theoretical, geometrical progression of values estimated by the expression $Q=K2^n$, where K is a constant for a species and n is an integer.

the nuclear volume, namely, as an elliptical cylinder and an ellipsoid (two thirds the volume of the elliptical cylinder). As calculated for an elliptical cylinder, the values are 9.3×10^{-12} g nucleic acid per nucleus in I and II combined, and 35×10^{-12} g in Class III and IV and V combined. There is, however, a trimodal distribution of values in the class of abnormal and cancer cells, as shown in Fig. 1, and the averages for the subgroups are 17, 38, and 67×10^{-12} g, respectively. A hypothetical envelope of points is given in Fig. 1 by normal frequency-distribution

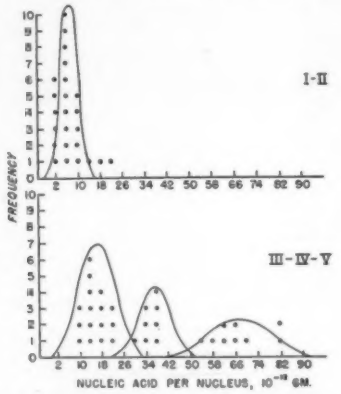

FIG. 1. Frequency of occurrence (ordinate) of certain quantities of nucleic acid per nucleus (abscissa) for cells of Class I and Class II combined (upper graph) and of Class III and Class IV and Class V combined (lower graph). Values computed for the nucleus as an elliptical cylinder. Each dot signifies an observation. Smooth curves have maxima at the average value for the class or the subgroup.

curves, which have maxima in the neighborhood of 9×10^{-12} g for Class I and Class II combined and at 18, 36, and 72×10^{-12} g, respectively, for the subgroups of Class III and Class IV and Class V combined. It can be computed from the data in Table 3 that the averages for values enclosed by a given curve in Fig. 1 differ from those of the adjacent distribution by five or more times the standard error of the difference. Thus, the distributions are statistically distinct.

When computed for an ellipsoid, the values are 6.2×10^{-12} g per nucleus in Class I and Class II combined, and 11, 25, and 45×10^{-12} g, respectively, for the subgroups of the abnormal and cancer cells. The frequency distribution curves for the envelope of points then have maxima in the neighborhood of 6, 12, 24, and 48×10^{-12} g, respectively.

If there is a geometrical progression in the average quantity, Q, of nucleic acid per nucleus in one cell-class as compared with another, the value, Q, may be represented by the expression

$$Q = K2^n,$$

where K is a constant and n is an integer. The relation and the agreement between the Q values, both as determined by microspectroscopy and as computed by the foregoing expression, are given in Table 3, where $n = 1$ for interphase normal epithelial cells and $n = 2$, 3, 4 for interphase squamous cancer cells. Despite the agreement, however, it must be emphasized in view of the askewness of the distribution of the points in Fig. 1 and because of the errors in cytometry and microspectroscopy that this may represent an oversimplification in the interpretation of the data.

An analogous expression for the average quantity, Q_{DNA}, of DNA per nucleus is given by

$$Q_{DNA} = K_{DNA}\, 2^n,$$

where K_{DNA} is a constant for the cells of a species and n is an integer. The value of K_{DNA} as derived from the literature is of the order of 3×10^{-12} g for mammalian cells (*1, 2*), and n has the value $n = 0$ for haploid (germ) cells; $n = 1$ for interphase diploid (somatic) cells; $n = 2$ for dividing diploid cells; and $n = 2, 3 \ldots$ for various orders of polyploidy. If a geometrical progression in the average quantity of DNA per nucleus does occur, it is quite possible that the frequency distribution of cells (both resting and dividing) with respect to the n values will be a characteristic expression of the pattern of growth of a tissue. The envelope of points for the tissue will be the integral, with respect to the n values, of the frequency-distribution curves of the component cells.

The geometrical progression in the total nucleic acid content of the interphase nucleus of the squamous cancer cell is consistent with the general concept of growth by reduplication and division (*46–49*). Since nucleic acids are prominent constituents of chromosomes (*20, 50–52*) and of nucleoli (*20*), the increase in the nucleic acid content of the single nucleus of the interphase cancer cell—or of any other cell—is conceivably related to the following mechanisms of growth: an acquisition of an even integral multiple of the fundamental number of chromosomes (polyploidy); a reduplication of the mass of individual chromosomes without division (endomitosis and polyteny); and a geometrical increment in the heterochromatin or in the nucleolar (RNA) apparatus. It is of interest in this regard that certain cancer cells of man are found to have, relative to normal cells, a two and four times greater volume of the nucleus (*36*), the mass of which has long been held to be related to the number of chromosomes (*46*); a two to four times greater volume of the individual chromosomes (*38*); an even multiple of the basic number of nucleoli (*38*); even, odd, and nonintegral multiples of the fundamental chromosome number (*29, 30, 53–55*); and many morphological abnormalities of the chromosomes (*29–30, 53–55*). It is intriguing to think that the nucleic acids themselves may perpetuate these alterations in the cell. Aside from such considerations, the observations reported herewith have tangible bearing upon the cytological diagnosis of cancer.

Upon the completion of the foregoing study, it was apparent that there were two conditions which ideally

should be, but were not, fulfilled in this or in contemporary microspectroscopic work of a similar kind: (*a*) the nuclei should be optically homogeneous, and (*b*) the nuclei should be randomly studied. Utilizing mechanical procedures for the homogenization and the conversion of tissues into suspensions of nuclei and with controlled conditions of mounting, it has been possible in recent work to fulfill conditions (*a*) and (*b*). The nuclei are optically homogeneous as shown by phase and interference microscopy which measure differences in optical path of the order of 0.02 μ. A preliminary comparison of nuclei so derived from normal cells and cancer cells of certain tissues of the mouse indicates a difference in nucleic acid content substantially similar to that reported herewith for a tissue of the human being.

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News and Notes

Wildlife Disease Association

In conjunction with the seventeenth North American Wildlife Conference, held in Miami, Fla., March 17–19, a number of investigators met informally and officially organized the Wildlife Disease Association. During the previous year a preliminary draft of a statement of scope and purpose had been prepared by Drs. Bell, Cheatum, and Jellison. This was circulated among other members of a committee, and their suggestions were incorporated into the statement where it seemed justified during discussion at the Miami meeting. The revised statement is as follows:

The practice of wildlife management in North America has attained the status of a recognized profession. As professions go, it is a youthful one, but the responsibilities with which it is charged are heavy. It is asked to manage desirable populations of wildlife species so that they may be at least maintained and, in many instances, increased beyond their current numbers. On the other hand, the profession is charged with the control of nuisance species whose populations are believed to conflict so excessively with human health and economy, or with the welfare of more desirable species of wildlife, that their reduction appears warranted.

Much more knowledge than is currently available is required for judicious handling of many of the problems incident to managing wildlife populations. There are natural limitations to the abundance of all species, limitations that are affected by climate, food, and natural enemies.

Among the natural enemies, diseases are of great im-

portance in their effect on wildlife populations. This phase of wildlife research needs more attention and coordination than it has received heretofore. The identification, epizoology, and significance of diseases are subjects of investigation by private, state, and federal agencies. Some of these agencies are primarily concerned with wildlife diseases as they bear on public health, others as they may affect animal industry. State and federal conservation agencies are often primarily concerned with disease factors limiting wildlife resources. The importance of some of the disease agents to the public health, the livestock industry, and the populations of wild animals has already been demonstrated. A closer working relationship is needed between all the investigators whose work is contingent on wildlife disease problems. The Wildlife Disease Association has been formed to achieve this coordination among fields, including population dynamics, nutrition and nutritional diseases, animal pathology, bacteriology, virology, parasitology, immunology, toxicology, therapeutics, and related subjects.

The association should eventually consider ways and means of effectively advising on the organization of wildlife disease studies in areas not adequately serviced for this type of work and to provide information on the kinds of assistance now available from diagnostic laboratories.

It is the intention of the association to establish a newsletter or some other form of communication for disseminating matters of concern to members of the association and other interested agencies or persons.

Scope and purpose of the association shall be to: (1) compile a directory of workers and their specialties in this field; (2) channel pertinent information on current studies and developments to members of the group; (3) provide a means for dissemination of information to organizations concerned with wildlife management, public health, and animal health; (4) provide opportunities for discussion of mutual problems through an annual meeting held in conjunction with the North American Wildlife Conference.

It is planned that a full day of informal discussion will be arranged in conjunction with the eighteenth North American Wildlife Conference, which is to be held in Washington, D. C., March 9–11, 1953. The Wildlife Disease Association will probably meet on March 8.

Anyone interested in becoming affiliated with this association should communicate with Carlton M. Herman, Patuxent Research Refuge, Laurel, Md., or with one of the other members of the committee.

J. F. BELL	W. L. JELLISON
W. B. BELL	N. D. LEVINE
E. L. CHEATUM	M. N. ROSEN
I. McT. COWAN	C. M. HERMAN, *Chairman*

Scientists in the News

William Benesch, graduate physicist at Johns Hopkins University, has been awarded a fellowship by the Belgian-American Educational Foundation, to work in the laboratories of the Astrophysical Institute at Liége. He will also visit other institutes in France, Italy, Germany, and England, consulting with astrophysicists interested in his field.

Lewis K. Dahl has joined Brookhaven National Laboratory as chief of the Medical Research Service in the hospital. He was formerly assistant physician in the hospital of the Rockefeller Institute for Medical Research in New York City.

Maurice J. Day, metallurgical engineer at U. S. Steel Co., Pittsburgh, has been named manager of the materials and processes division at Armour Research Foundation of Illinois Institute of Technology. He succeeds **W. A. Casler,** who has been acting manager of the division since May 1951. Mr. Casler continues as assistant director of research and manager of program development.

George Glockler, head of the Department of Chemistry and Chemical Engineering at the State University of Iowa, has been awarded the 1952 Iowa Medal of the American Chemical Society's Iowa Section. Now on leave from his academic post, Glockler is director of the science division of the Office of Ordnance Research, Durham, N. C. The Iowa Award, consisting of an engraved medal, is presented annually to an Iowa chemist or chemical engineer for meritorious achievement in fundamental or applied chemistry.

C. Gilbert Goss, development engineer in electronic circuitry at Oak Ridge National Laboratory for the past several years, has joined Radiation Counter Laboratories, Inc., as director of electronic research.

John D. Green, formerly of Wayne University College of Medicine, has joined the Department of Anatomy, University of California School of Medicine at Los Angeles.

Samuel C. Harvey, professor of surgery in oncology at the Yale School of Medicine, and **Roscoe H. Suttie,** professor of civil engineering in the School of Engineering, have retired from active teaching at Yale University. Dr. Harvey became resident surgeon in 1919 at the New Haven Hospital and also instructor in surgery at the Yale School of Medicine. In 1924 he was appointed William H. Carmalt professor of surgery, the chair he held until his appointment in oncology in 1947. In his retirement, Dr. Harvey will be William H. Carmalt professor emeritus of surgery. Professor Suttie started as an instructor in civil engineering in 1913 and, after spending a year as assistant engineer in the U. S. Geological Survey, returned to Yale as assistant professor of civil engineering and became a full professor in 1944. His chief interest has been sanitary engineering.

Carl F. Holden (USN, ret.) has been elected president of Federal Telecommunication Laboratories, Inc., Nutley, N. J., research unit of International Telephone and Telegraph Corporation. **Harold H. Buttner,** who had been president of Federal, will assume duties as vice president of I. T. & T., coordinating the research and development activities of the parent company.

Former President **Herbert Hoover** has received the award "Engineer of the Century" from the Northwest

Engineering Centennial. Mr. Hoover has been a member of the AAAS for half a century, having joined the Association in 1902.

John L. Kask, assistant director of the Fish and Wildlife Service, has been appointed a U. S. member of the Inter-American Tropical Tuna Commission and the International Commission for the Scientific Investigation of Tuna, succeeding **Milton C. James,** who retired as assistant director on March 31. Dr. Kask has recently attended the fourth meeting of the Inter-American Tropical Tuna Commission held in San José, Costa Rica, Aug. 13–15.

Garth S. Kennington has joined the faculty at Lawrence College as assistant professor of biology, replacing **Robert M. Chew,** who has been appointed an instructor in zoology at the University of Southern California.

Merle Lawrence has been appointed associate professor of physiologic acoustics, Department of Otolaryngology, University of Michigan Medical School. He has been associate professor of psychology, Princeton University.

The Damon Runyon Memorial Fund for Cancer Research, Inc., has made a fellowship grant to **Gladys M. Mateyko,** a research assistant in the biology department laboratories at New York University. Her research will center on the single cell and specific cellular areas that are isolated for individual study. Her work is aimed at the eventual diagnosis of cancer from a single cell.

Paul W. Merrill is retiring after a 33-year astronomical career in Pasadena. Dr. Merrill is a staff member of the Mount Wilson and Palomar observatories, operated jointly by California Institute of Technology and the Carnegie Institution of Washington. He joined the Mount Wilson staff in January 1919. Since 1939 he has been editor of the observatory publications, and in 1949 he was appointed a member of the Observatory Committee, which determines scientific policies for both Wilson and Palomar. He was a councilor and vice president of the American Astronomical Society, and has been president of the Astronomical Society of the Pacific.

Inger Onshuus, Farmaceutisk Industri, Oslo; **Mahir Colakoglu,** University of Ankara, Turkey; **Perttie V. Laakso,** University of Helsinki, Finland; and **M. de Barros Ferraz,** Instituto Agronômico of Campinas, São Paulo, were recent visitors at the Eastern Regional Research Laboratory, Philadelphia.

The Ebert Medal for distinguished pharmaceutical research was presented to **Lloyd M. Parks,** of the University of Wisconsin School of Pharmacy, at the American Pharmaceutical Association's centennial convention in Philadelphia. Dr. Parks has been a member of the Wisconsin faculty since 1938 and this year is chairman of the A.Ph.A. Scientific Section.

The magazine *Archivos de Medicina Internacional,* published by the Washington Institute of Medicine for doctors in Mexico, Spain, and all the countries of South America, has devoted a recent issue to the work of **Leonard J. Ravitz,** of Duke University, on electrical measurements and treatment in mental illness. The feature article was entitled "Fenómenos electrocíclicos y estados emocionales."

William Reiner-Deutsch has been invited to deliver a lecture before the Ophthalmological Society of the East of France in December. Dr. Reiner-Deutsch is director of laboratory research, Department of Research, New York Eye & Ear Infirmary.

Henry T. Skinner has been appointed director of the National Arboretum, Washington, D. C. Dr. Skinner, for the past 12 years curator of the Morris Arboretum in Philadelphia, succeeds **B. Y. Morrison,** who retired this past year.

Royal W. Sorensen, professor emeritus of electrical engineering, has retired from California Institute of Technology after a 42-year career, the longest service record of any Caltech faculty member. He plans to continue his high voltage equipment research and will also supervise the construction of 200,000-ampere current-testing equipment, being built on the campus to his design.

Adolph J. Stern, chairman of the Department of Chemistry at Wagner College, has been appointed dean of the college, following a ten-year association with Wagner. In 1942 he was appointed instructor in the department, and in 1950 he was chosen chairman. He has also been director of the summer session since 1947.

Roger W. Truesdail, president, Truesdail Laboratories, Inc., Los Angeles, has been invited to address European university and technical groups in Oslo, Bergen, Stockholm, Gothenburg, Copenhagen, Amsterdam, Brussels, Paris, and London. His subject will be "The Role of Commercial Laboratories in Industrial Research in the USA" and "Recent Developments of Food Technology in the USA."

Education

On behalf of the **Foundry Educational Foundation,** a committee of 19 foundry specialists and educators will serve as advisers to Illinois Institute of Technology during the coming year. The committee will advise engineering students on their scholastic programs and will carry out other local functions of the foundation, which was established in 1947 by the casting industry to improve educational offerings in the field. H. A. White, manager of the Smeeth-Harwood Company, Chicago, is chairman of the committee.

New York University College of Engineering has renamed its Department of Meteorology the Depart-

ment of Meteorology and Oceanography. New curricula will stress physical oceanography and the relationships between oceanography and meteorology. G. Neumann, former oceanographer at the University of Hamburg, has recently joined the department.

The **University of North Carolina** School of Medicine has made the following staff appointments: R. A. Ross (obstetrics and gynecology); Edward C. Curnen (pediatrics); Ernest Wood and Charles A. Bream (radiology); R. Beverly Raney (orthopedic surgery); Thomas W. Farmer (neurology); Louis G. Welt and John T. Sessions, Jr. (medicine); Edward C. Frank and David R. Hawkins (psychiatry); Paul L. Bunce (surgery).

The **University of Tennessee** Medical Units in Memphis have appointed the following new staff members: Yo Seup Song, of Seoul; Pierre-Antoine Finck, of the University of Geneva; Bengt Larsson, of Malmo, Sweden; Luis Prieto, Jr., of City Hospital, Cleveland; Helen Prieto, of St. Luke's Hospital, Cleveland; W. S. Gilmer, of the University of Virginia; and Tom D. Norman, of the USAF Hospital, Sheppard AFB, Wichita Falls, Kan. All the new staff members will be instructors in pathology except Dr. Prieto, Jr., who will be an assistant professor.

The **University of Texas** has appointed Edwin J. Fellows, director of biological sciences of Smith, Kline & French Laboratories, to the position of visiting lecturer in pharmacology and toxicology. Dr. Fellows will retain his position with SKF during the 12 months of his lectureship.

Grants and Fellowships

The following industrial scholarships have been awarded at **Illinois Institute of Technology**: Ceco Steel Products: Edward Bedrosian and Bert Bergstrom, engineering; Transparent Package: Alan Grossberg and Fred C. Hardtke, chemistry; La Salle Steel: John C. Krone; Witco Chemical: Theodore Lewis; Foundry Educational Foundation: Donald R. Adolphson.

The **John and Mary R. Markle Foundation** is continuing for the sixth year its Scholar in Medical Science program, in which grants of $30,000 at the rate of $6,000 per year are made for the support of each scholar. The number to be named in 1953 has not been determined, but 21 were appointed in 1952. Each accredited medical school is invited to nominate one candidate, and all nominations should be in the hands of the foundation, 14 Wall St., New York 5, on or before *Dec. 1.*

The **National Paraplegia Foundation** has awarded the following fellowships for basic studies in spinal functions: Pei-chin Tang, of the University of Washington School of Medicine; Dominick P. Pupura, of Harvard University Medical School; and Ellis C. Berkowitz, of the University of California Medical School at Los Angeles. The foundation will also make a special grant to Donald Monroe, Boston City Hospital, to carry on a study of sex function in paraplegia.

The **National Science Foundation** graduate fellowship program for 1953–54 will follow the basic pattern set during the current year, in which nearly 600 fellows are receiving advanced training. The majority of awards will be made to graduate students, although a limited number will be made to postdoctoral students. Application forms, returnable by *Jan. 5,* will be available after *Oct. 1* from the foundation, Washington 5, D. C. No awards will be made for study in clinical medicine, although grants will be made to medical students interested in careers in medical research.

Among **Ramsay Memorial Fellowships** recently awarded was one to David R. Lide, of Harvard, at the University of Oxford. The awards, which are all tenable in British universities, included two British fellowships, one Canadian, one Spanish, and one Swiss. Three fellowships were renewed.

Schenley Laboratories has made a grant of $17,000 to the Langbord Virus Laboratory at Hahnemann Medical College and Hospital for antivirus research under B. A. Briody, recently appointed director of the laboratory. Wayne University has received $4500 from Schenley for special research in organic chemistry and natural products, and the University of Wisconsin has received $3600 for fermentation research.

In the Laboratories

The **Atomic Energy Commission** will construct a new gaseous diffusion plant for the production of uranium 235 in Pike County, Ohio, about 22 miles north of Portsmouth. Preliminary design calls for an estimated cost of about $1,200,000,000, and up to 400,000 kw of power, which will be supplied from existing facilities. Later operations will require 1,800,000 kw. Kenneth A. Dunbar has been named area manager for the construction and operation, and R. H. McCulloh deputy area manager.

Baxter Laboratories, Inc., has appointed Fred H. Schultz head of the Department of Physiology-Pharmacology and director of pharmacological research. Harry L. Fevold has been appointed head of the Department of Biological Chemistry and director of biochemical research, and Leonard G. Ginger head of the Department of Organic Chemistry and director of organic chemical research. Myron Usdin has been made executive officer of the Department of Bacteriology. Other recent appointments to the staff of the Scientific Division are Arthur Berger and Bernard C. Wexler.

The **Communicable Disease Center,** USPHS, Atlanta, Ga., has appointed Donald S. Martin, former dean of the University of Puerto Rico School of Medicine, chief of the Bacteriology Laboratory. He succeeds Martin Frobisher, Jr., who has resigned to become chairman of the new Department of Bacteri-

ology at the University of Georgia. John M. Henderson, until recently professor of sanitary science in the Columbia University School of Public Health, has been appointed a consultant to the Vector Control and Investigations Branch.

Meetings and Elections

The **Analytical Section** of the International Union of Pure and Applied Chemistry has elected the following officers: president, C. J. van Nieuwenburg, of Delft (1951–53); vice presidents, I. M. Kolthoff, of Minneapolis (1951–53), and P. E. Wenger, of Geneva (1951–55); secretary, S. E. Q. Ashley, of Pittsfield (1951–55). They constitute the Executive Committee. A Section Committee is responsible for the general conduct of activities, and is made up of the following membership: the officers of the section; the president, secretary, and treasurer of the union; and F. E. Beamish, G. Charlot, R. C. Chirnside, F. Feigl, J. Gillis, and J. I. Hoffman. Commissions on Analytical Reactions, Microtechniques, Physico-Chemical Data of Analytical Interest, and on Terminology and Expression of Analytical Results are carrying on the work of the section with the help of eight subcommissions.

The tenth annual **Pittsburgh Diffraction Conference** will be held at Mellon Institute of Industrial Research, Pittsburgh, Nov. 6–7. Technical sessions on Instrumentation and Methods, Neutron Diffraction, and General Diffraction Studies and a symposium of invited papers in the field of Order-Disorder Studies are being arranged. For further information and a copy of the program, address E. E. Wiker, U. S. Steel Company, 234 Atwood St., Pittsburgh 13, Pa.

Recent Deaths

Angel A. Aballi (71), pediatrician, Havana, July 22; **F. A. Abbiati** (47), of Longmeadow, Mass., a vice president of Monsanto Chemical Company, Boston, Aug. 13; **Ward P. Anderson** (50), chemical engineer, Larchmont, N. Y., Aug. 12; **Harry Aranow** (73), obstetrician and gynecologist, New York, July 31; **Carl Beck** (88), surgeon, Sayre, Pa., July 21; **Clarence H. Bellinger** (65), neurologist and psychiatrist, Brooklyn, N. Y., Aug. 12; **H. A. Black** (79), physician, Pueblo, Colo., Aug. 7; **Lloyd A. Blanchard** (58), safety engineer, Washington, D. C., July 26; **Adelaide L. Brewster** (51), educational film expert, New York, Aug. 11; **Caxton Brown** (73), electrical engineer, Summit, N. J., July 25; **Oscar Brunler** (58), chemist, physician, and physicist, Santa Barbara, Calif., Aug. 1; **Margaret Byington** (—), sociologist, Chatham, Mass., Aug. 17; **Frederic Grosvenor Carnochan** (61), of New City, N. Y., biologist, Suffern, N. Y., Aug. 3; **Rose Cohen** (57), of New York, dentist, Philadelphia, Aug. 12; **Paul A. Collins** (67), opthalmologist, New York, Aug. 12; **Melville T. Cook** (83), of Baton Rouge, La., plant pathologist, Washington, D. C., Aug. 11.

Bernhard Dattner (65), neuropsychiatrist, New York, Aug. 12; **Lincoln Davis** (80), surgeon, Needham, Mass., July 29; **Frederick J. De Natale** (47), psychiatrist, Poughkeepsie, N. Y., Aug. 3; **Charles W. Dowsett** (77), metallurgist, Toronto, Aug. 18; **Lammot du Pont** (71), former president, E. I. du Pont de Nemours & Co., Fishers' Island, Conn., July 24; **Harry E. Ehrlich** (43), surgeon, New York, July 29; **Mark Fishberg** (55), urologist, Brooklyn, N. Y., Aug. 19; **Ralph W. Gallagher** (71), former president, Standard Oil Company (New Jersey), New York, July 31; **Robert L. Gray** (71), physician, Philadelphia, Aug. 11; **William T. Griffiths** (57), metallurgist, Redhill, Eng., July 30; **M. E. Haas** (45), president, Bussey Products Co., Chicago, June 13; **John P. Hall** (56), of New York, mining engineer, Chicago, Aug. 14; **Andrew J. Higgins** (65), inventor, New Orleans, Aug. 1; **Otto K. Hunerwadel** (60), agriculturist, Rangoon, Burma, July 30; **Andrew Hutton** (63), of Groton, Conn., design and development engineer, Westerly, R. I., Aug. 1.

P. J. Iverson (69), educator, Fargo, N. D., Aug. 12; **John D. Jungman** (76), physician, Philadelphia, July 24; **Edward M. Keefe** (83), physician and surgeon, Cincinnati, July 21; **H. A. Kohman** (71), research chemist, Pittsburgh, Aug. 16; **Harland A. Ladd** (48), educator, Augusta, Me., July 20; **Searle H. Lanyon** (56), physician, Philadelphia, Aug. 11; **Clinton C. Lawrence** (59), plant pathologist and forester, Stamford, Conn., Aug. 18; **George C. Low** (78), expert on tropical diseases, London, July 31; **James F. McDonald** (75), neuropsychiatrist, New York, Aug. 15; **Mary W. Marvell** (80), physician, Fall River, Mass., Aug. 9; **R. B. Mildon** (74), former vice president, Westinghouse Electric Corporation, New York, Aug. 4; **George I. Miller** (81), surgeon, Brooklyn, N. Y., July 29; **Albert J. Newlin** (64), meteorologist, San Jose, Calif., Aug. 14; **Boris Nikitin** (—), chemist, Moscow, July 23; **Carl M. Nye** (78), engineer, Chatham, Mass., July 27; **William A. Orton** (63), of Northampton, Mass., economist, Damariscotta, Me., Aug. 13; **Newdigate M. Owensby** (69), psychiatrist, Atlanta, Aug. 10.

Leonard Peckitt (92), industrialist and chemist, Allentown, Pa., July 21; **William R. Redden** (71), former national medical officer, American Red Cross, Brooklyn, N. Y., Aug. 11; **Edith Rockwood** (—), sociologist, Washington, D. C., Aug. 17; **William T. Salter** (50), pharmacologist, New Haven, Conn., July 30; **Frank J. Sensenbrenner** (87), of Kimberly-Clark Corporation, Neenah, Wis., July 22; **David Sheitlis** (—), pathologist, New York, Aug. 11; **Henry J. Sherman** (82), civil engineer, Moorestown, N. J., July 29; **Joseph Skapier** (48), cytologist, New York, Aug. 15; **Charles S. Smith** (65), physiologist, San Marcos, Tex., Aug. 1; **Arthur K. Stone** (90), tuberculosis specialist, Framingham, Mass., July 31; **Charles B. Sullivan** (79), psychiatrist, Boston, July 23; **Rueben S. Tour** (62), chemical engineer, Cincinnati, Aug. 1; **G. Harold Warfield** (59), of New Rochelle, N. Y., engineer, Vineyard Haven, Mass., Aug. 6; **Harry D. Watts** (67), engineer, Southampton, N. Y., July 27; **W. Lawrence White** (44), botanist, Concord, Mass., July 31; **Virgil Zener** (74), educator, Reading, Pa., Aug. 12.

Technical Papers

Sex Ratio of Offspring from Irradiated Male Mice[1]

H. Kalmus,[2] J. D. Metrakos, and M. Silverberg

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A group of interrelated phenomena (visible mutations, sperm lethality, semi- and total sterility, reduction of litter size, etc.) resulting from irradiation of male or female mice has been reported by Snell (*1–4*), Hertwig (*5–7*), Russell (*8*), and by many others in this field. In the present paper some preliminary data on the alteration of the sex ratio of offspring produced by irradiated male mice are presented.

Littermates of the highly inbred A/HeJax strain of mice were paired and mated when 60 days old. The first and second litters were weighed, sexed, and discarded. One or two days before the birth of the second litter, the male was separated from the female. Within 24 hr of the birth of the second litter the genitalia of the male parent were irradiated with an x-ray tube rendering a dose of 150 or 300 r. Immediately after irradiation the paired mice were reunited. Although some differences were found between the two dosages, in this brief report only the pooled results will be presented. The second litters and those litters that were born within 40 days after irradiation of the male constitute the "control" and "experimental" groups, respectively. Although the first litters are usually below par both as to number and weight, the second and third litters are quite comparable (*9*).

Twenty pairs of mice produced three successive litters, the third one within 40 days of irradiation of the male. The mean litter size (± SE) and sex ratio of the 60 litters are given in Table 1. It will be noted that the mean litter size of the third litters (5.95 ± 0.31) is significantly lower ($P = < .001$) than that of the second litters (7.65 ± 0.22). Such a reduction in litter size has been reported previously (*1*) and appears to be proportional to the dosage. However, it is here further noted that the mean reduction of litter size (1.70) is largely due to the mean reduction of females (1.45).

TABLE 1

MEAN LITTER SIZE (± SE) AND SEX RATIO OF THE FIRST THREE LITTERS OF 20 PAIRS OF A/HEJAX MICE, AND A STATISTICAL COMPARISON OF SECOND AND THIRD LITTERS

Litter order	No.	Females	Males	Total	Sex ratio
First	20	3.05 ± 0.27	3.35 ± 0.26	6.40 ± 0.34	1.10
Second	20	3.80 ± 0.20	3.85 ± 0.21	7.65 ± 0.22	1.01
Third	20	2.35 ± 0.24	3.60 ± 0.27	5.95 ± 0.31	1.53
3rd/2nd%		61.8%	93.5%	77.8%	151.5%
Student's t		4.64	0.73	4.47	
P (38 d.f.)		$< .001$	.5	$< .001$	

TABLE 2

MEAN LITTER SIZE (± SE) AND SEX RATIO OF THE FIRST THREE LITTERS OF 39 PAIRS OF A/HEJAX MICE, AND A STATISTICAL COMPARISON OF SECOND AND THIRD LITTERS

Litter order	No.	Females	Males	Total	Sex ratio
First	39	3.18 ± 0.23	3.44 ± 0.18	6.62 ± 0.24	1.08
Second	39	3.64 ± 0.21	4.03 ± 0.22	7.67 ± 0.19	1.11
Third	39	3.67 ± 0.24	4.02 ± 0.23	7.69 ± 0.29	1.10
3rd/2nd%		100.8%	99.8%	100.3%	99.1%
Student's t		.09	.01	.02	
P (76 d.f.)		.9	.9	.9	

This alteration of the sex ratio between the control and experimental litters appears to be a real one, for whereas the difference between the mean number of males in the control (3.85 ± 0.21) and experimental litters (3.60 ± 0.27) is not significant ($P = .5$), that between the mean number of females in the same two groups (3.80 ± 0.20 and 2.35 ± 0.24) is highly significant ($P = < .001$).

As litter size and sex ratio are extremely difficult characters to analyze, the present data were compared with a second and more independent "control" group. Table 2 gives the mean litter size (± SE) and sex ratio for the first three litters of 39 A/HeJax mice living in the laboratory during the course of this experiment. The main point to be noted here is that there is no significant change ($P = .9$) in the sex ratio (1.11

[1] The authors are indebted to C. B. Peirce, of the Department of Radiology, Royal Victoria Hospital, for technical guidance and use of radiological equipment.

[2] Present address: Galton Laboratory, University College, London, England.

and 1.10) or litter size (7.67 ± 0.19 and 7.69 ± 0.29) between the second and third litters. Furthermore, the sex ratio of both the second and third litters is not significantly different from that of the first litter (1.08).

The material is too small for any detailed analysis and cannot be compared at the present time with the results of Hertwig (*6*), who used larger dosages. No attempt, therefore, will be made at this time to interpret the above results; however, several theories have been proposed, and experiments are now being conducted in order to test these theories.

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The Inhibition of Blood Clotting *in Vitro* and *in Vivo* by Tyrosinase

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Mushroom tyrosinase has been found to inactivate several biologically active proteins such as invertase, pepsin, trypsin, chymotrypsin, and insulin (*1–3*) and more recently has been shown to inactivate thrombin and to so modify fibrinogen that with thrombin it clots very slowly to form a clot which is decreased in amount and amorphous instead of fibrous in structure (*4*). In view of the marked effect of tyrosinase on thrombin and fibrinogen, it became a problem of considerable interest to study the action of tyrosinase on the clotting system of whole blood and plasma. Tyrosinase was also injected into the blood stream of mice in order to investigate *in vivo* effects of tyrosinase on the blood clotting mechanism of animals.

Nine ml human[2] blood was obtained by venipuncture and mixed with 1 ml 0.1 *M* sodium oxalate. To 2 ml blood was added 0.2 ml tyrosinase (Treemond, 10,000 Miller-Dawson u/ml or 1500 u/mg protein), and the system was incubated in a water bath at 37°. A control was run simultaneously which contained tyrosinase inactivated by boiling. At successive intervals aliquots were removed for the measurement of clotting time and added to an equal volume of 0.02 *M* $CaCl_2$ in a small test tube at room temperature. The tube was tilted at intervals, and the clotting time was taken as the time required for the blood to cease to flow when the tube was tilted (*5*). The inactivation by tyrosinase of one or more components of the clotting system is shown in Fig. 1. The exponential character of the curve for inactivation is very similar to that described for the action of tyrosinase on thrombin or fibrinogen (*4*).

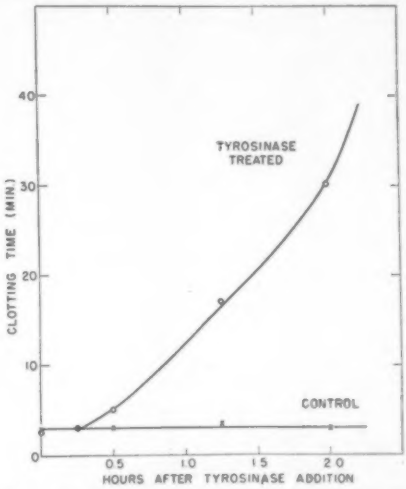

Fig. 1. Action of 0.2 ml tyrosinase on the clotting mechanism of 2.0 ml human blood at 37°. The control contains inactivated tyrosinase.

The inactivation of the clotting system of whole human blood is independent of the presence of the blood cells, since results similar to those of Fig. 1 were obtained with plasma prepared by centrifuging the above blood sample or with reconstituted lyophilized human plasma (Sharpe & Dohme).

In order to study the effects of tyrosinase on the clotting mechanism *in vivo* the enzyme was injected intracardially into laboratory white mice (either Harvard or Swiss strain) weighing about 25 g. For injection purposes the tyrosinase was dissolved in 0.9% NaCl. As in the *in vitro* studies, inactivated tyrosinase was used in the control experiments. Blood samples from the tip of the tail were drawn into fine capillary glass tubes at successive intervals for measurement of clotting time. Two different methods for measuring the rate of clotting were used. In the first, clotting time was taken as the time required for the column of blood to cease to flow in the capillary when gently moved to and fro by variations in air pressure. The second method was the standard capillary tube technique for measuring the rate of clotting (*6*) and involved breaking off pieces from the tube at successive intervals and measuring the time required before a fibrin thread forms when the broken pieces are slowly separated. Neither method was ideal, and somewhat variable results were obtained, especially with samples of blood that required a long time to clot. This difficulty was related to the fact that tyrosinase-treated

[1] Aided by a grant from Ethicon Suture Laboratories.

[2] I.W.S., Type B, Rh positive.

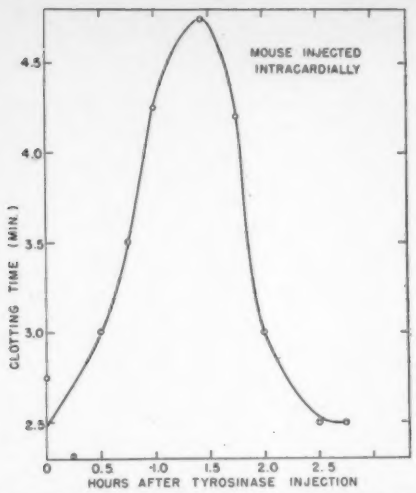

FIG. 2. Effect of 0.2 ml tyrosinase, injected intracardially in a 25-g mouse, on the clotting system of the blood. Blood samples were removed at successive intervals from the tip of the tail for measurement of clotting time.

blood not only clotted slowly but never produced as firm and rigid a clot as did normal blood.

Typical changes in clotting time resulting from the injection of 0.2 ml tyrosinase into a 25-g mouse are shown in Fig. 2. Although the clotting time is about doubled within 1.5 hr after the injection, the clotting mechanism returns to normal in 3 hr. In a series of experiments the clotting time showed a two- to four-fold increase within 1.0–1.5 hr after the tyrosinase was injected. The restoration of a normal clotting system in the treated mouse was often delayed for 8 or even 24 hr in some experiments. Animals which showed the greatest effect of tyrosinase on the blood often appeared sick and occasionally died in 24–48 hr. In control animals which were injected with boiled tyrosinase the clotting system was unaffected during the 8 hr of the experiment.

The problem of immunization of mice to tyrosinase was investigated by using a single animal for a series of injections over a period of 1–2 months. Results with several animals clearly show that injections of tyrosinase cause the production of antibodies which inhibit the enzyme *in vivo* (*7*). For example, in one mouse the clotting time increased to 11 min when tyrosinase was injected the first time; 15 days later when it was injected again the increase was up to 5 min, and 8 days later a third injection did not noticeably change the clotting time from the normal value of 2.6 min.

These studies show that tyrosinase rapidly inactivates the clotting system of plasma, whole blood, and of circulating blood of the mouse. In the living animal the clotting system returns to normal after several hours. Experiments are in progress to determine which components of the clotting system of blood *in vivo* and *in vitro* are inactivated by tyrosinase and to determine whether the eventual restoration *in vivo* of the system to normal represents a reversal of the inactivation or a replenishment by the blood-synthesizing organs of the inactivated components.

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The Nonsurgical Treatment of Cataract[1]

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Because of the high incidence of cataracts and the hazards associated with their surgical removal (disregarding psychological and economic factors), a nonsurgical treatment of cataracts has been sought by ophthalmologists for many years. At one time it was thought that the administration of bovine lenticular extracts might be of therapeutic value (*1*), but this was not established (*2*). Subsequently, and because of the differences that were noted between lenticular extracts from fish and the proteins of mammalian lenses (*3–5*), the possibility of fish lens proteins being of therapeutic value was considered.

In November 1950, experimental work was started to study the ocular changes in rats subjected to high dietary stresses. The results, as yet unpublished, cover more than a year's work on the lens changes in rats under controlled conditions. Groups were large enough (24 or more) to compensate for individual biological differences. Selected groups of individually caged rats were placed under a physiological stress such that changes might, and did, occur in the eyes. These eye changes were ophthalmoscopically observed and critically described each week. Concurrently, blood and urine chemistry, employing microtechniques supplemented by paper chromatography, was carried on. Initial experiments were performed on rats 6 weeks of age; but before the work was completed, not only were all age groups studied, but stresses applied to females at time of breeding were finally evaluated, in order to differentiate between the effect on the offspring during fetal life and during lactation, by examining the eyes of baby rats at weaning. These experiments, which involved many hundreds of rats whose eyes were examined ophthalmoscopically weekly, ultimately made it possible to induce cataracts

[1] The patients were treated at the office of Dr. Ginsberg, 705 New Jersey Ave., Brooklyn, N. Y.

nt will, the degree of opacification being a function of age or stress, or some combination of age and stress. Obviously, the development of a bio-assay technique will be of tremendous value in future studies.

Recently, in a large series of experimental animals, controlled induced cataracts were caused to regress to disappearance by the parenteral administration of fish lens protein extracts. Such cataracts could not again be reinduced experimentally with the original stress if, concurrently, fish lens protein extracts continued to be administered. As a result of these experiences, we decided to administer a fish lens protein extract to human cataractous patients. The results obtained have to date been so promising that it was thought advisable to make this preliminary report.

The lenticular extracts are prepared by comminution of the lens tissues, their extraction with a physiologically compatible solvent, the removal of insoluble residues, and the sterilization of the final solution. Total protein concentrations are less than 2% as determined by the usual Kjeldahl procedures: the pH is approximately 7.4; and although several thousand injections of the fish lens extract have been administered, no allergic reactions have been observed. Empirically, a dosage of 1.0 ml administered thrice weekly has been standard with all cases discussed in this preliminary report. Thirty or more times this dose (i.e., 0.5 ml/kg body weight for rats, given every day for several weeks) has produced no discernible untoward effects.

The first group, totaling 14 patients, has now had four or more subcutaneous injections of this extract. The degree of visual impairment has ranged from patients who had been unable to carry out their normal household duties for several years, or had been unable to move about the streets unattended, to patients manifesting only visual fogging. In all cases, after four injections patients have reported subjective improvement. Objectively, after six or more injections administered three times weekly, a diminution of lens opacities has been observed ophthalmoscopically (Figs. 1 and 2). As a result of this treatment in several cases who have had 30 injections, the progress has been from a completely incapacitated individual to one with practically normal visual acuity. Most of the patients have been between 60 and 70 years old, and in most cases the cataracts have been typical senile opacities. Several of the patients had cataracts associated with diabetes and were hypertensive, or arteriosclerotic, or had combinations of these conditions. In all cases there has been a clearing of lens opacities, and retinal detail, which previously could not be seen, can now be studied with the ophthalmoscope.

Concurrently with these preliminary clinical trials, biophysical studies are being made that indicate tremendous differences between the proteins of the lens of the mammalian eye and those of fish lenses. The ultracentrifuge and the electrophoretic apparatus have been employed in these studies, and for the first

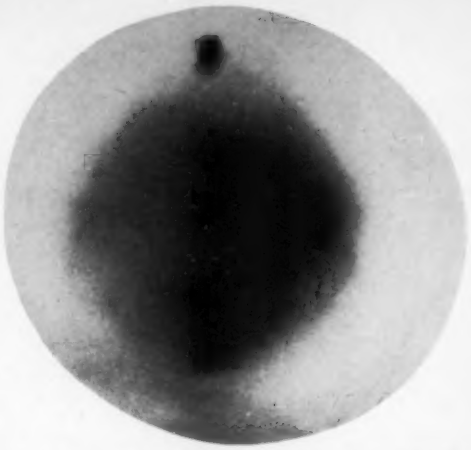

FIG. 1. Patient L: original ophthalmoscopic photograph of appearance of cataract in right eye.

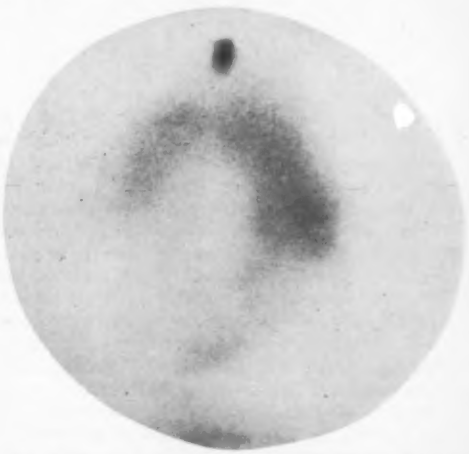

FIG. 2. Patient L: ophthalmoscopic photographic appearance of right eye after about two month's treatment.

time information on the distribution, molecular weight, and electrophoretic motilities of lenticular proteins has been obtained. Essentially the pattern that in the past was applied to blood plasma studies has been followed. It is expected that not only will the components of fish lenses be fractionated and characterized in the customary terms of electrophoretic purity, molecular weight and dimensions, configuration, electrical charge, etc., but that these fractions will be evaluated, utilizing the rat bio-assay already mentioned, in terms of biological activity. They will be published as soon as the data can be correlated, and the necessary calculations made.

Results in an additional 12 cases, selected more or less at random from nearly 100, which are to be the

TABLE 1

Patient	Age	Visual acuity		Dates (1952)	No. injections
		O.D.	O.S.		
Mrs. S. W.	34	Light and dark	20/20–3	Feb. 27	
		20/40	20/20–3	July 9	40
Mr. S. S.	64	20/100	20/200	Feb. 28	
		20/30	20/50	Apr. 29	29
Mrs. E. D.	54	Light and dark	20/100	Feb. 24	
		20/100	20/30	May 21	40
Mr. M. F.	67	20/60	Amblyopic	Jan. 9	
		20/15		Mar. 6	22
Mrs. G. R.	49	20/150–1	20/30–2	Feb. 18	
		20/40–3	20/15–3	Apr. 29	30
Mrs. S. J.	54	20/200	20/150	May 12	
		20/200	20/40+	July 16	28
Mrs. M. M.	60	Aphakial	20/150	Jan. 22	
			20/60	Mar. 19	30
Mrs. C. P.	70	20/70	20/80	Apr. 23	
		20/30	20/30	July 9	36
Mr. P. de P.	39	20/50–2	> 20/200	Apr. 3	
		20/20+	20/100	June 25	41
Mrs. I. L.	57	20/70	20/30	Apr. 30	
		20/40	20/30	May 28	12
Mrs. J. A.	67	Fingers at 5 ft	20/80	May 21	
		20/100	20/40	July 16	26
Mrs. P. B.	70	20/80	20/60	Jan. 2	
		20/20–2	20/20–1	Apr. 7	34

subject of an extensive and more detailed clinical report, are shown in Table 1. No attempt is made to amplify the mitigating factors that affect visual acuity, such as retinopathy, which is not discovered until a clearing of the lens permits a detailed examination of the fundus; nor are the sketches and written descriptions of the ophthalmoscopic appearances of the opacities included. These data, as well as all other pertinent observations regarding coexisting pathology, such as diabetes, hypertension, etc., and subjective changes reported by the patients, have been a normal part of the critical evaluation procedures employed. For the purpose of this brief and preliminary paper, a record of the initial and final visual acuities, together with the ages of the patients and the number of injections received, should answer that first and most important question that will be asked in regard to this nonsurgical therapy of cataract: "Is the patient's vision improved?"

Another clinical series involving approximately 50 patients is being observed and studied under conditions of the most critical scrutiny, and these results will be submitted for publication as soon as available. In view, however, of the promise that this therapy has shown so far, this report is submitted in order that those interested in the subject may be advised of the work that is in progress.

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Observations on a Chlorophyll-Deficient Strain of *Chlorella vulgaris* Obtained after Treatment with Streptomycin

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It has been demonstrated that streptomycin brings about a loss of chloroplasts in the flagellate *Euglena* (*1*) and in the seedlings of certain Gramineae, and that it causes the loss of color in crown gall tumors of carrot (*2*). Therefore, research was recently initiated to determine whether streptomycin would induce the loss of chloroplasts in algae other than *Euglena*. *Stichococcus bacillaris*, *Scenedesmus dimorphus*, and two species of *Chlorella* (sp. and *vulgaris*) were investigated. All these species are capable of heterotrophic growth in the dark, with glucose as a carbon source.

Vegetative cells of all these algae were treated in tubes containing Maerten's solution ($Ca(NO_3)_2 \cdot 4 H_2O$, 1.0g; $MgSO_4 \cdot 7 H_2O$, 0.2g; K_2HPO_4, 0.2g; 1 ml of trace element solution [*3*]; and distilled H_2O, 1000 ml), 0.5% glucose, and four concentrations of streptomycin sulfate (500, 2500, 5000 and 7500 µg/ml). One

triplicate set of cultures was incubated in a dark cabinet at 22–24° C, and the other set under fluorescent light at 450 ft-c at the same temperature.

Sample loopsful were taken from the tubes at intervals of 1 hr, 30 hr, 65 hr, and 168 hr, streaked on 2% agar plates of Maerten's solution plus 0.5% glucose, and incubated in the dark cabinet.

A straight-line relationship between streptomycin concentration and killing, as well as between duration of treatment at a given concentration and killing, was observed. *Scenedesmus*, *Stichococcus*, and *Chlorella* sp. were similarly affected by the drug, showing few surviving cells after 30 hr. *Chl. vulgaris* was least sensitive to streptomycin, a few cells remaining viable after 65 hr in the lowest concentration in the dark.

The fact that the longest survival time (65 hr) was found with cells incubated in the dark is consistent with the larger number of colonies appearing from the dark-incubated tubes in all cases.

Appearing among the colonies on the 65-hr plate just discussed was a colony that was half yellow and half green. When cells from the yellow half were examined, they also proved to be *Chlorella*, so they were streaked on Maerten's-glucose plates, incubated in the dark, and studied further.

Cells of the "mutant"[1] grown in the dark are slightly smaller and more ovoid (4.0μ × 4.6μ) than the wild type (4.6μ in diam). They appear colorless under the microscope, and their chloroplasts are slightly distorted. The dark-grown colonies are yellow to light-green and have never been observed to vary in color or shape during numerous plating experiments. The wild type retained its original color through three dark transfers extending over two months.

The behavior of the "mutant" is entirely different in light and dark. Although it is yellow-green in the dark, in contrast to the dark-green wild type, it is dark-green in the light and capable of growth on a medium devoid of carbon source.

All transfers of the dark-green photosynthesizing cells of the "mutant" give rise to yellow-green colonies in the dark, thus indicating that this is a stable mutant.

Preliminary determinations of the absorption spectra of methyl alcohol extracts of the pigments in the Beckman spectrophotometer indicate that, although the light-grown cells have an absorption spectrum similar to that of the wild type in light and dark, chlorophyll *a* is lacking in the dark-grown mutant. There is also evidence that a compound similar to protochlorophyll is present.

Although wild types of *Chlorella* possess the same pigments in light or dark (*4*), mutants have been obtained which do not synthesize chlorophyll (*5*). One of these mutant strains (*6*), which accumulates Mg vinyl phaeoporphyrin, greens in light as does the "mutant" described here. It has not yet been determined whether this "mutant" accumulates the same compound, or whether there is a blocking at the next stage as postulated by Granick (*5*); i.e., that of formation of chlorophyll *a* from protochlorophyll.

[1] There is a possibility that this form is not a mutant in the usual sense of the word: that is, that its nuclear genes have not been altered. If streptomycin brought about some defect in the chloroplast, and if the chloroplast were a self-duplicating unit, this would be a cytoplasmic mutation. It is equally likely, however, that a true genetic mutation has occurred and that it was picked up by chance during the scanning of a large population.

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Effect of Prior Injection of Non-Mouse Tissues on Growth of Tumor Homoiografts in Mice[1]

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With the exception of the so-called nonspecific transplantable tumors, grafts of mouse tumors do not survive when the donor and host animals are from genetically unrelated inbred lines. With certain experimental procedures, however, the normal resistance of an animal to a tumor homoiograft (i.e., grafts of a tumor indigenous to one inbred line into mice of a genetically unrelated inbred line) can be broken down. This can be effected either by the prior injection into the host of lyophilized normal or cancerous mouse tissues (*1–4*), fresh tissue homogenates (*5*), tissue antiserums (*6*), frozen tumor tissue (*7, 8*), or by repeated inoculations of the foreign tumor (*9*). As a rule, the best results follow the administration of normal tissues from animals of the inbred line to which the donor graft is indigenous, or the injection of homologous tumor tissue. "Cross reactions," however, are occasionally obtained when normal or cancerous tissues from an inbred strain to which the donor tumor is not indigenous are used (*3, 10*).

The present paper is directed to the question of species specificity of the effect. Three different sets of experiments are reported, dealing with the results of injection of lyophilized tissues from rats, hamsters, and guinea pigs on mouse tumor homoiografts.

The test tumor used was the tumor 15091a, an anaplastic mammary gland carcinoma that is indigenous to the inbred A strain of mice. It grows rapidly, on subcutaneous inoculation, in 100% of strain A animals of both sexes, killing most of the hosts within 3–5

[1] Supported in part by a grant-in-aid from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council, and in part by a research grant from the National Cancer Institute, National Institutes of Health, USPHS.

weeks after implantation. The test hosts were mice of the inbred strains C57BL/6Ks (a C57 black subline) and C57BR/edJax (a C57 brown subline). These strains are not related to the A strain. The recipient mice were about equally divided by sex, and ranged in age from 2 to 4 months at the start of the experiment. All appeared healthy and vigorous.

Liver, kidney, and spleen were taken from rats, hamsters, and guinea pigs. The tissues were secured under sterile conditions, frozen over dry ice within 1 hr after being excised, and immediately thereafter subjected to freeze-drying. The lyophilized tissues were powdered and stored in ampoules sealed under vacuum. For purposes of injection, the tissues were ground in glass homogenizers in a vehicle of sterile double-distilled water. Injections of the resultant suspensions were given intraperitoneally twice weekly in the amount of 0.5 ml/injection, containing 10 mg dry weight of tissue. Control groups of mice received injections of lyophilized homologous tissue or saline.

One week after the last injection, single inoculations of bits of minced live tumor were made by trocar, under sterile conditions. The grafts were placed subcutaneously in the suprascapular region. The subsequent course of growth of the implants was followed by palpation. Animals were classified as "takes" if they died with progressively growing tumors, and as "negatives" if there was no sign of a graft for at least two consecutive months.

The data are presented in Table 1. It is apparent that only the prior injections of the lyophilized homologous mouse tumor tissue abrogated the resistance of the hosts to the homoiografts. The numbers of "takes" in the groups injected with lyophilized guinea pig kidney or spleen are not statistically significant, as compared with the saline-injected controls.

TABLE 1

EFFECT OF PRIOR INJECTIONS OF LYOPHILIZED TISSUES ON THE GROWTH OF TUMOR HOMOIOGRAFTS IN MICE

Substance injected	Total mg/mouse (dry wt)*	Mice dying with tumors†	
		C57BL/6Ks	C57BR/edJax
Rat tissue			
Liver	100	—	0/17
Kidney	100	—	0/19
Spleen	100	—	0/10
Saline (control)	(5 ml)	—	0/20
Hamster tissue			
Liver	100	0/19	—
Kidney	100	0/20	—
Spleen	100	0/5	—
Saline (control)	(5 ml)	0/10	—
Guinea pig tissue			
Liver	50	0/23	0/23
	100	0/22	0/15
Kidney	50	1/22	0/18
	100	0/19	2/9
Spleen	100	1/6	—
Saline (control)	(2.5 or 5 ml)	0/24	1/25
Homologous tumor	50	9/10	7/10

* Injections given intraperitoneally twice weekly in 10 mg/injection.

† Numbers in numerators are the numbers dying in each experimental group; numbers in denominators are the total numbers of mice in each group.

The failure of the out-of-species tissues to abolish the resistance of the host to the homoiografts indicates that we are dealing with a specific reaction evoked by substances, present in both the lyophilized mouse tissues and the fresh tumor inoculum, which must have moieties in common. This supposition is further supported by the predominant absence of cross reactions (*3, 7, 8, 10*) when tissues from inbred mouse strains not homologous to the tumor inoculum are used.

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The Role of Darkness in Sexual Activity of the Quail[1]

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Studies with plants have demonstrated that the duration of the night or dark period is an essential factor in photoperiodism (*1*). The requirements of some plants for short day lengths for flowering were shown to be constituted of two parts: (*a*) a requirement for a specific minimum amount of light followed by (*b*) a specific minimum of darkness (*2*). The possible role that periods of darkness may play in the photoperiodic responses of higher animals has not been conclusively shown.

Evidence that the dark period is critical to the photoperiodic responses of lower animals has been obtained in two instances. Shull (*3*) has shown that wing production by aphids is dependent upon a requirement for a light period followed by a long dark period, entirely comparable to the plant requirements mentioned above. Jenner (*4*) has found that reproduction in the pulmonate snail (*Lymnaea palustris*) requires long photoperiods, but short photoperiods with brief light interruptions of the night will bring on reproduction.

[1] Journal Paper No. 606 from Purdue University Agricultural Experiment Station in cooperation with the Indiana Department of Conservation.

[2] We wish to thank J. H. Martin and B. B. Bohren for their generous cooperation in making available facilities of the Poultry Husbandry Department for this study.

Through the manipulation of daily light duration a photoperiodic control of sexual activity has been found in birds (*5*). Although much has been said of light as a stimulus, little or no importance has been accorded dark as a discrete factor in the photoperiodic responses of birds. Marshall (*6*) partially reviewed the literature of photoperiodism in birds and found many points of criticism of experimental methods. He states, "Before it can be unquestionably accepted that light fluctuation causes . . . prenuptial recrudescence, far more elegant experimental techniques than those used in the past will have to be devised."

Reproduction in bobwhite quail (*Colinus virginianus*) is brought on by long days and is entirely prevented if day lengths are kept short (*7*). The object of the present study was to determine whether sexual activity could be induced by changing the length of the dark period without changing the hours of light given.

Bobwhite quail hatched in August 1951 were transferred to indoor cages on January 22, 1952. Five groups of 6 pairs each were given artificial light treatments, each pair being subjected to light from a 40-w incandescent lamp. One group received a long day (17 hr), one group a short day (10 hr), and three groups were on a short day of 9 hr with a 1 hr interruption during the dark period. These interruptions, given at different times for each of the three groups, provided for maximum dark periods of 7, 10½, and 12½ hr. Temperatures in the cages ranged from 75° to 100° F.

After 37 days the birds were sacrificed. Full sexual activity, evidenced both by ovulation (or ripe follicles) and free spermatozoa, was obtained in all groups receiving either long days or short days with night interruptions. In contrast the group receiving short days with no interruption of the dark period showed no reproductive development. Average gonad and oviduct weights are presented in Fig. 1. In other experiments

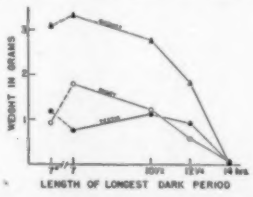

FIG. 1. Influence of length of dark period on weights of reproductive organs of the quail. Asterisk indicates 17-hr day. All other treatments received 10 hr of light/day.

(which will be described more fully later) we have obtained full sexual activity in both sexes with 30 min interruption and in the male with 15 min interruption when the basic light ration was 10 hr.

From the present experiment it is clear that 10 hr of light including a night interruption can evoke full sexual activity in the bobwhite quail, whereas the same light ration as a single daily exposure cannot. It can be concluded that both the light period and the dark period are involved in sexual activation of this species.

Fig. 1 reveals the role of the dark period. In the four short-day groups, each receiving 10 hr of light in each 24-hr cycle, the tissue weights are approximately inversely related to the length of the longest dark period. Since full sexual activity or no sexual activity could be obtained with the same number of light hours by varying the length of the dark period, it is evident that the dark period is an inhibiting factor.

This situation is comparable to the behavior of long-day plants. Under normal environmental conditions, reproductive responses both in quail and long-day plants are inhibited by long nights. If a short-day, long-night is given, a negative response results, but reproductive activity begins if the night is interrupted. It is apparent that the duration of the dark period is a major controlling factor of photoperiodic responses of plants, some lower animals, and of at least one higher animal—the bobwhite quail.

We believe that the demonstrations by Shull (*3*) and by Jenner (*4*) are the only reliable demonstrations of the role of the dark period in animals. Hart (*8*) was not conclusive in attempting to show that estrus is produced in ferrets by breaking the dark period, since his sexually activated group received both a night interruption and extra artificial light, neither of which was applied to the unactivated controls. It is highly probable that the ferret *is* responsive to the night interruption, like quail, but this was not unequivocally shown.

For the male starling, Burger (*9*) reported 12½ hr as the minimum light quantity for complete spermatogenesis and, with co-workers, later (*10*) obtained spermatogenesis with flashing light when the total light quantity ranged from 7.3 to 10 hr, but these investigators did not observe the implication that the length of the dark period is critical to the photoperiodic stimulus.

In fowls, increased egg production is obtained when a light period is given in the middle of the night (*11*), even when the light is of very short duration but of great intensity (*12*), but no attempt has been made to initiate sexual activity by these means, nor to clarify the role of the dark period per se.

It would appear that the present demonstration with bobwhite quail is the first clarification of the importance of the dark period in the photoperiodic response of any higher animal.

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The Effect of Terramycin and Aureomycin on Blood Coagulation[1]

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Within recent years, with the rapid development of new antibiotics and the widespread practice of their almost routine administration, there has been an increased interest in their effect upon the coagulability of blood and thromboembolic phenomena (*1*). Published data, experimental and clinical, both support (*2–4*) and deny (*5–7*) the role of antibiotics as a causative agent in increasing the coagulability of the blood in patients receiving these drugs.

Large doses of the streptomyces-derived antibiotics (aureomycin used) could also affect the normal heparin content of the blood (*8*). This would have an effect that would not always be in the anticipated direction of increased coagulability, for heparin may normally function to regulate the elaboration of plasma thromboplastin, and, in the absence of the latter, decreased coagulability might ensue (*9*).

Macht and Farkas (*4*), working with rabbits and observing them over a 3-hr period, reported that aureomycin caused an increase in the coagulability of the blood. Shapse and Wright (*7*) in this clinic noted no alterations of clinical importance in the blood coagulation time of 30 patients after the administration of aureomycin.

The question of embolism is of such great surgical importance that we checked the experimental work of Macht and Farkas on the effect of aureomycin on the blood coagulation time in rabbits, and in the same study we included the effect of terramycin on the blood coagulation time in rabbits and on the bleeding, clotting, and prothrombin times in patients.

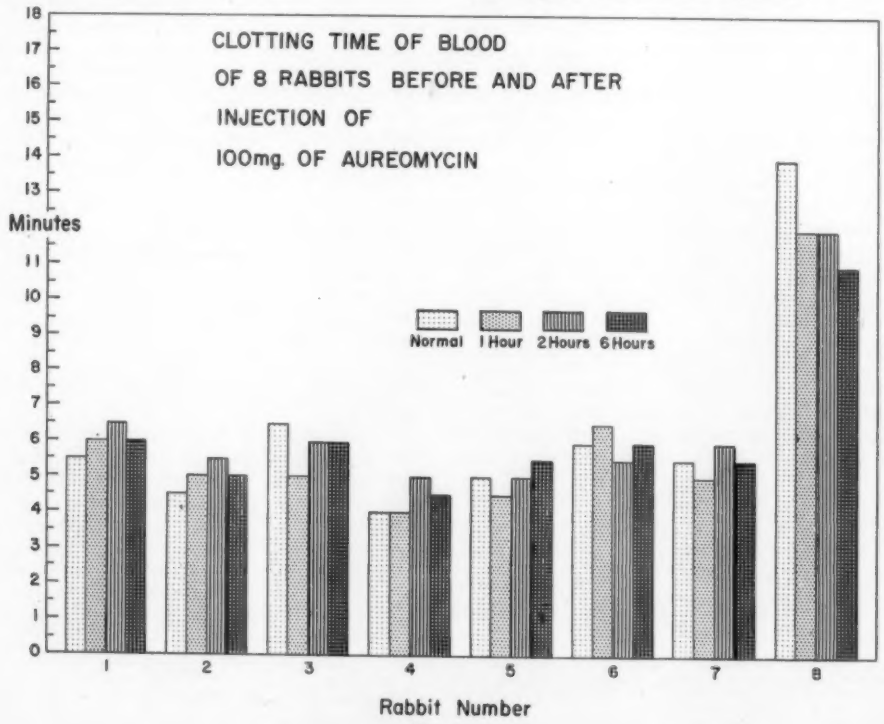

FIG. 1.

Experiments were conducted on 16 rabbits in which 8 were given 100 mg aureomycin glycinate intravenously, and 8 were given 100 mg terramycin glycinate intravenously. The drugs were dissolved in 2 ml distilled water and injected into the marginal veins. Blood specimens were obtained by both marginal vein and intracardiac punctures in various animals. Clotting times were done before the first injection and again at 1-, 2-, and 6-hr intervals after administration of the antibiotics. The clotting times were done by the

[1] Aided by grants from Chas. Pfizer & Co., Brooklyn, N. Y., and the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y. Terramycin was furnished through the courtesy of Chas. Pfizer & Co., and aureomycin through the courtesy of the Lederle Laboratories Division, American Cyanamid Company.

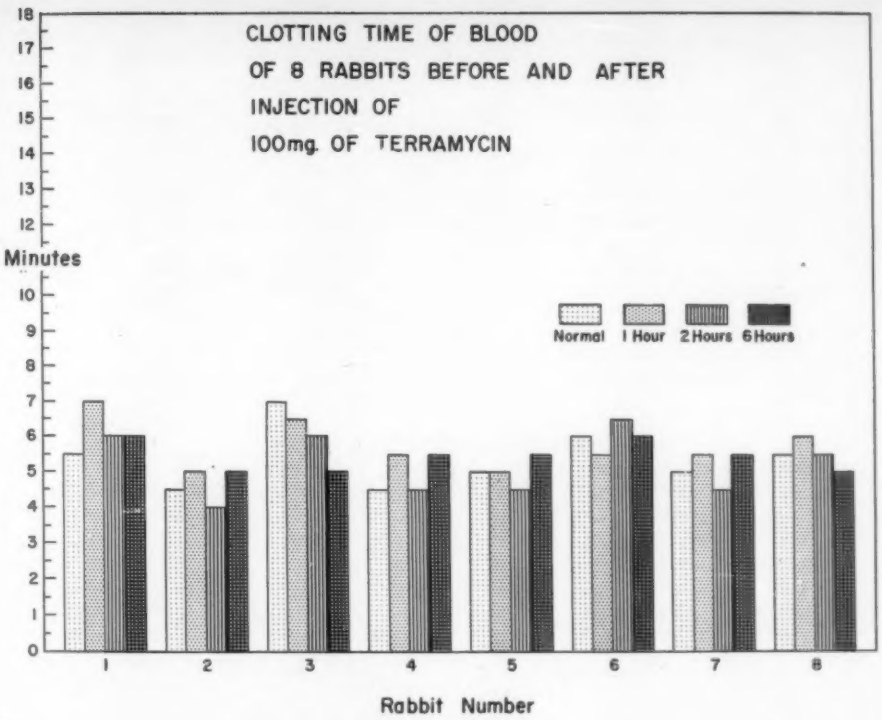

FIG. 2.

method of Lee and White. One ml of blood was placed in each of three tubes (10 mm in diam), which had been rinsed with normal saline. The tubes were inverted at 30-sec intervals until the clot could not be displaced.

The results in each rabbit are expressed graphically in Figs. 1 and 2. Fig. 3 presents the average alteration in clotting time according to the time interval after administration of each drug.

Bleeding and clotting times were done on 30 patients before and at 1-, 24-, and 48-hr intervals after the initiation of terramycin therapy. Eleven patients received 500 mg terramycin intravenously every 12 hr, and the remainder received 250 mg of terramycin orally every 6 hr. The bleeding times were determined by puncturing the ear lobes of the patients with a #20-gauge needle and blotting the area with filter paper every 30 sec until bleeding ceased. The clotting times were determined by the method of Lee and White. In 16 patients concurrent prothrombin times by the method of Quick were done before and at 24- and 48-hr intervals after institution of terramycin therapy.

The cases used were all adults and consisted of the following diagnoses: Cellulitis of extremities, 5; breast abscesses, 3; infected lacerations, 3; appendicitis, 3; human bites, 2; infected 2° and 3° burns, 2; ruptured peptic ulcers, 2; penetrating stab wounds of the abdomen, 2; and one each of the following: compound fracture of tibia, thrombophlebitis of leg, cervical cellulitis and adenitis, periurethral abscess with urinary extravasation, cystitis and pyelonephritis, ischiorectal abscess, incarcerated inguinal hernia, and acute orchitis. Table 1 shows the average bleeding, clotting, and prothrombin times and their variations at the indicated times.

TABLE 1

AVERAGE BLEEDING AND CLOTTING TIMES OF 30 PATIENTS AND AVERAGE PROTHROMBIN TIMES OF 16 PATIENTS ON TERRAMYCIN THERAPY

	Av bleeding time (min)	Av clotting time (min)	Av prothrombin time (sec)
Pretreatment	3.40	8.37	17.10
1 hr	3.63	8.63	
24 "	3.60	8.48	16.90
48 "	3.51	8.68	17.10

Although there were some individual variations in the clotting times in the rabbits, and in the bleeding, clotting, and prothrombin times in the patients, there were no significant changes in any of these tests. Re-

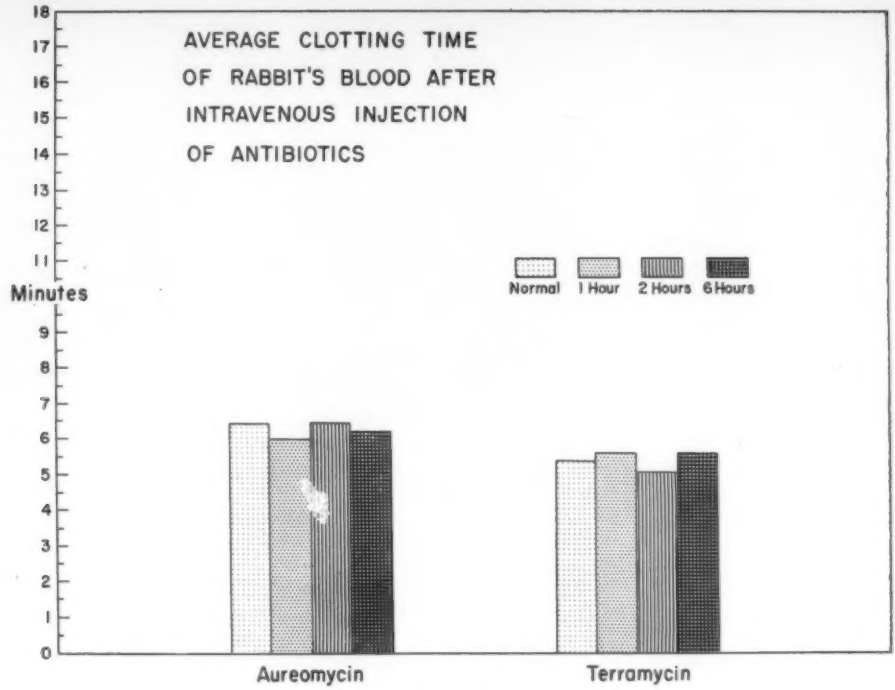

FIG. 3.

cent articles (*9, 10*) have shown terramycin to have no appreciable effect on the prothrombin time of human blood.

We have treated several hundred patients with both aureomycin and terramycin and have noted no increased frequency of embolism. Thus we feel that neither aureomycin nor terramycin in the recommended dosages produces any alteration in the blood coagulation mechanism that is of any clinical significance, and that neither drug should be withheld from any patient because of the fear of producing an intravascular clot.

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Comments and Communications

The Scientist as Citizen

I WAS gratified to read in Comments and Communications (May 16, 1952) "A Citizen's Duty," by Paul D. Foote, of Gulf Research and Development Company, urging that national meetings of scientific, technical, and trade associations be arranged so that they do not fall on election day. Mr. Foote wisely says, "The votes of the readers of this journal, who represent a group of the highest intelligence, are especially desirable;" and again, "Why not recognize our obligations as citizens and, throughout the country, arrange all association meetings on dates that will not conflict with voting?"

In the same issue of SCIENCE, Kirtley F. Mather, who discusses "The Problem of Antiscientific Trends Today," says,

> Scientists, by the very nature of their mental habits, are internationalistic rather than chauvinistic in their outlook. [He adds that] Scientists, moreover, are naturally devoted to the principles of democratic freedom that shine so clearly in our constitutional Bill of Rights, based as they are upon the one most important freedom of all, the freedom to think one's own thoughts and to express them so that they may be appraised in the court of public opinion.

Assuming that one's own country is worth while, one cannot be soundly "internationalistic" without being truly patriotic in the effort to protect and preserve our own country. The international situation has so deteriorated that the first thing on the agenda is to do one's best to appraise it correctly and act wisely. The danger is a real and present one.

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β-Glucuronidase and Glucuronide Synthesis

IN a recent review of the relationship between estrogens and enzymes, Fishman (*1*) discusses his hypothesis that the enzyme β-glucuronidase acts synthetically in the animal body. It therefore seems appropriate at the present time to recount briefly the principal evidence for the existence in the animal body of a glucuronide-synthesizing system, distinct from β-glucuronidase.

Fishman's hypothesis was originally put forward to explain his discovery (*2–4*) that the β-glucuronidase activity in nonsex organs, such as liver, rises in response to the administration of menthol or borneol, whereas the activity of the enzyme in uterus and vagina is increased by administration of esteriol or other estrogens. The complete explanation rests on three distinct postulates: (*a*) that the enzyme acts synthetically *in vivo*, (*b*) that the tissue activity responds adaptively to the presence of excess substrate, and (*c*) that the enzyme differs in specificity or function according to its location in the body. Without accepting the others, we shall limit ourselves here to considering the first and key postulate.

β-Glucuronidase is present in nearly all animal tissues that have been studied (*5*), and its activity is measured *in vitro* by following the hydrolysis of biosynthetic glucuronides (*6–8*); its activity in certain organs can be made to rise or fall *in vivo* by administering a variety of both glucuronidogenic and nonglucuronidogenic agents (*2–4, 9–11*); certain glucuronides cause a rise in activity (*4, 9*); *in vitro* this enzyme is powerfully inhibited by saccharate (*12–15*), an effect that has recently been shown to be due to saccharo-1:4-lactone present as impurity in the saccharate solution (*16, 17*); it is not inhibited by fluoride, iodoacetate, dinitrophenol, or sulfate (*12, 18*).

The activity of the glucuronide-synthesizing system can be measured in tissue slices and appears to require oxidation and phosphorylation; this enzyme system has been found only in liver and, to a minor extent, in the kidney (*19–21*); its activity in liver is unaltered by treating animals with agents that cause changes in liver β-glucuronidase activity (*20*); *in vitro*, glucuronide synthesis is not inhibited by saccharo-1:4-lactone (*14, 17*), but it is inhibited by anaerobiosis, cyanide, iodoacetate, fluoride, azide, dinitrophenol, and sulfate (*19, 21*); glucuronic acid in low concentration does not stimulate glucuronide synthesis by liver slices, nor does it overcome depression due to various causes (*19, 21*); in high concentration, glucuronic acid itself inhibits glucuronide synthesis *in vitro* (*21*), as it does β-glucuronidase (*12, 15*).

From their study of phenol conjugation by tissue slices (*22*), De Meio and Arnolt arrived at certain conclusions regarding glucuronide synthesis that are contrary to some of the points mentioned above. It was later shown, however, that their method did not measure conjugation of phenol with glucuronic acid (*23*), a finding that has been accepted by De Meio (*24*).

Florkin, Crismer, Duchateau, and Houet (*25*) obtained evidence for every slight condensation of glucuronic acid with borneol after prolonged incubation of the two compounds together in high concentration in presence of β-glucuronidase, but they considered that it did not follow that glucuronides are synthesized by this mechanism *in vivo*. Using a very sensitive color reaction for *o*-aminophenylglucuronide (*23*), Karunairatnam and Levvy (*12*) could find no condensation of *o*-aminophenol with glucuronic acid in presence of β-glucuronidase or crude liver homogenates. Under suitable conditions, any hydrolytic enzyme should theoretically be able to catalyze the reverse reaction, but several instances are known where resynthesis in the body proceeds by quite a different mechanism.

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SINCE the important contributions of Levvy and Kerr have been repeatedly discussed (*1–3*), this comment is merely intended to restate our attitude toward the concept of β-glucuronidase as a catalyst of the synthesis of glucuronides and to clarify—if it is possible—the prevailing viewpoints on glucuronide synthesis.

With regard to the first consideration, although direct proof that the enzyme catalyzes the conjugation of glucuronic acid *in vivo* is lacking, this mechanism is at least theoretically possible and there are good reasons for its existence (*1–3*). This concept became the basis of a working hypothesis proposed in 1940 (*4*) in an attempt to explain certain glucuronidase phenomena in animals, and it has been helpful in suggesting new experiments and has led to the discovery of new information. It will remain a useful *ad hoc* hypothesis until it has outlived its value for our purposes, or until future work suggests a better explanation of the role of the enzyme.

It is Levvy's implied contention that certain of his *in vitro* data negate the validity of the working hypothesis which we favor. It should be pointed out, however, that the translation of *in vitro* data into valid deductions *in vivo* is always hazardous even with the use of surviving tissues, unless the *in vitro* findings do coincide with observations in the whole animal. In our view, the findings in the whole animal are more readily explained on the basis of a predominately synthetic action of β-glucuronidase. The weight that one wishes to attach to negative *in vitro* results is a matter of personal opinion.

With respect to the second consideration, two mechanisms for glucuronide synthesis have received attention. The first entails the synthesis of glucuronides from three carbon precursors without the participation of glucuronic acid itself. A requirement of oxidation and phosphorylation is stated by Lipschitz and Bueding (*5*), Karunairatnam *et al.* (*6*), and Storey (*7*). One suspects that the processes of oxidation and phosphorylation concern the synthesis of glucuronic acid only and not its conjugation. The other concept holds that glucuronic acid itself is the immediate conjugating compound and that the enzyme β-glucuronidase participates in the conjugation (*4*).

This second mechanism is supported in part by direct experimental evidence in the intact animal. Thus, King (*8*) and Packham and Butler (*9*), using radioactive carbon, have shown that the synthesis of borneol glucuronide in the guinea pig and of naphthyl glucuronide in the rat takes place with the preformed molecule of glucuronic acid. Glucuronic acid, administered intraperitoneally, was incorporated into biosynthetic naphthyl glucuronide (*9*) at twenty times the rate of any three carbon precursors.

This observation does not rule out the possibility that there may be other mechanisms of glucuronide synthesis not requiring preformed glucuronic acid, nor does it necessarily prove that β-glucuronidase catalyzes the conjugation. It does lend added support to the working hypothesis discussed here.

It is hoped that the exposition of these varying viewpoints concerning a biologically important phenomenon may benefit other investigators who may undertake work in this field.

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Book Reviews

Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve. 2nd ed. D. J. Finney. New York–London: Cambridge Univ. Press, 1952. 318 pp. $7.00.

The first edition (reviewed in SCIENCE, **107**, 76, [1948]) has proved very useful and was sold out some time ago. The second edition is nearly the same in content, but somewhat extended, with better paper and printing. The jacket and preface list the new material and the sections that have been changed.

The section on alternative distributions has been broadened, and discusses use of the angle transformation. The sections on maximum likelihood estimates, on the Parker-Rhodes equations, and on probits for quantitative response are revised to use a simplification of equations recently obtained by the author himself.

A new chapter has been added. It is not unified as

are the others, but discusses several recent developments in the field. It includes alternatives to maximum likelihood, situations where the population treated is estimated rather than known, experimental designs, independent action in mixtures, special methods for estimating high percentage points, and interpretation of standard errors.

The appendix on derivations has been considerably rearranged, and two new tables are added—on weighting with estimated populations, and on analysis of inverse sampling. The bibliography is extended. The book will continue to be almost indispensable in dosage-mortality studies.

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Wavelength Tables of Sensitive Lines. L. H. Ahrens. Cambridge, Mass.: Addison-Wesley, 1951. 86 pp. $3.00.

These tables are of great value for the analyst dealing with the spectrographic d-c arc analysis of compound materials such as rocks, soils, and minerals. The main table contains the wavelengths of the most sensitive lines of 68 elements and information about relative intensities and sensitivities, and excitation potentials. The most valuable feature of the book is the listing of all possible interfering lines of other elements occurring in the neighborhood of each sensitive line within the range of about ±0.5 A. Usually only three or four sensitive lines are listed—a sufficient number for an unambiguous identification. A smaller separate table lists the most sensitive lines of the rare earth elements and gives the values of the relative sensitivities as determined by Meggers and Scribner, and Smith and Wiggins.

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Proceedings of the Second Clinical ACTH Conference*; Vol. 1: *Research*; Vol. 2: *Therapeutics. John R. Mote, Ed. New York: Blakiston, 1951. Vol. 1: 531 pp.; Vol. 2: 716 pp. $8.50 each.

The rapid advances in medical science in recent years and the great increase in research have strained existing media for publication. Prompt announcement of significant discoveries, so important to continuing progress, is possible with relatively few journals, and it has become customary to read lay accounts months before the appearance of the scientific publication. The printing in book form of the proceedings of informal conferences has been tried as a partial solution to this difficulty. The careful correction and editing of such transactions is time-consuming, and often the better part of a year, or even longer, is required to produce the volume. On rare occasions, the printed transactions are ready for the reader as early as four months after the conference, and such was the case in this instance.

The two volumes comprise a record of a meeting held under the auspices of Armour and Company. The editor's introduction and the complete table of contents appear in each volume. The 102 contributions—41 in Volume I and 53 in Volume II—are by 312 authors; they cover a wide field, and nearly all branches of medicine are represented. The influence of corticotropin (ACTH) on various phases of metabolism and the effects of the hormone on a great variety of disease processes are the subjects of most of the papers. The communications largely represent new material destined for later publication in various journals. Most of the papers are followed by discussions, a total of 177 individuals thus participating, some as many as ten times. The contributions vary widely in quality and significance: some are products of precise and careful investigation; some recount large and others small series of observations; sometimes the conclusions are conservatively drawn, and at other times they reflect the enthusiasm of the investigators. Therapeutic effects might be graded as definite, slight, and negative by one observer, whereas the corresponding responses might be classed as dramatic, moderately dramatic, and disappointing by another.

The two volumes contain a wealth of detail on the physiological and clinical effects of the hormone newly introduced into therapeutics. Probably in no other single publication could one acquire information on so many aspects of the subject, and it is unfortunate that no index is provided. Without it the work does not serve well as a source of reference; although the table of contents lists the titles of the papers, there is no way of finding material within the extensive discussions. As there are some 357 separate items of discussion, some of them as long as or longer than the papers being discussed, and as these of necessity usually appear in their order of presentation at the conference regardless of subject matter, the reader has no way of finding what he seeks short of leafing through 1247 pages.

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Scientific Book Register

Seaweeds and Their Uses. V. J. Chapman. New York–London: Pitman, 1952. 287 pp. Illus. $6.00.

Theory of Matrices. Sam Perlis. Cambridge, Mass.: Addison-Wesley, 1952. 237 pp. $5.50.

Ultraviolet Radiation. Lewis R. Koller. New York: Wiley; London: Chapman & Hall, 1952. 270 pp. $6.50.

A Hundred Years of Biology. Ben Dawes. London: Duckworth; New York: Macmillan, 1952. 429 pp. $5.00.

Solubilities of Inorganic and Organic Compounds: A Compilation of Solubility Data from the Periodical Literature. Supplement to 3rd ed. containing data published during 1939–49, inclusive. Atherton Seidell and William F. Linke. New York: Van Nostrand, 1952. 1254 pp. $12.50.

The Theory of Relativity. C. Møller. New York: Oxford Univ. Press, 1952. 386 pp. $7.00.

Flour for Man's Bread: A History of Milling. John Storck and Walter Dorwin Teague. Minneapolis: Univ. Minnesota Press, 1952. 382 pp. Illus. $7.50.

Association Affairs

Prize in the Social Sciences

A PRIZE of $1000 for an essay on theoretical sociology was offered in 1951 (SCIENCE, 114, 508 [1951]), but in the opinion of the judges none of the entries adequately met the conditions established for the award, and no prize was given. A prize of $1000 is now offered for 1952 for essays reporting upon inquiry into the theory of social processes and group behavior. The conditions of the competition are as follows:

A. The contribution should further the comprehension of the psychological and cultural behavior of human beings in contemporary society, with specific recognition of the space-time frame of reference. The descriptions and interpretations presented should be developed from carefully controlled observations of actual social situations.

B. The development should be along those methodological lines that have been most prominent in the established sciences. Special attention in the particular case should be paid to the manner of theoretical-factual construction that can be studied in the line of progress from Charles Darwin through C. S. Peirce, William James, and John Dewey.

C. Clear recognition and steady stress should be given to observation as taking place always under postulation, and to postulation as always arising out of prior observation. If this view is not held, its alternative should be made scientifically clear. The essay should foster liberation from traditional philosophic-linguistic conventions and from outmoded boundaries between different social disciplines.

D. The focus of attention should be the process of movement and change in society, the interactions between individuals and groups, and the adjustments and readjustments in their relationships that make for activity and development.

COMMENT

This is not a prize for *conventional* work in the social sciences, even when done by the best conventional technicians; nor is it a prize for *any* kind of original work, no matter how brilliantly dialectical it may be. Fundamentally, it is a prize to encourage procedures within the framework of the best-formulated evolutionary naturalism and experimental or operational logic of the past half-century or more.

Manuscripts will be judged by a committee of three persons who will be fully qualified to appraise material in this field, but who will not necessarily be sociologists. Entries should be sent to Howard A. Meyerhoff, Administrative Secretary, American Association for the Advancement of Science, 1515 Massachusetts Ave., N.W., Washington 5, D. C., not later than *October 31.* To facilitate the work of the judges, the *material should be submitted in triplicate,* and the name of the author should be omitted from the typed copy. Definite preference will be given to unpublished manuscripts or to essays especially prepared for this competition, but articles that have been published since December 1, 1951, will not be excluded from the competition merely by virtue of having been published. Announcement of the winner will be made at the Association's St. Louis meeting in December, but the Committee of Judges reserves the right to withhold the prize if no worthy essay is submitted.

AAAS Research Grants

THE AAAS administers the A. G. Stillhamer Endowment Fund, which was given for the purpose of supporting research in biology. For several years the income from this fund has been allowed to accumulate, and the Association is now prepared to offer a $500 Stillhamer Grant for Biological Research in 1952. In subsequent years it will offer comparable grants of $500 in other scientific fields.

In addition to the Stillhamer Grant of $500, the Grants Committee will also consider applications for an award not to exceed $500, preferably in a field of science other than biology. This special grant is available for 1952 only, and it is not planned to offer it annually.

It is proposed to announce the 1952 awards at the St. Louis meeting of the Association, December 26–31, 1952, and applications will be received through *November 15.* Each application should provide the following information: name and address of applicant, his institution, a detailed description of the research project, together with a statement regarding any projects thus far made, the specific purpose or purposes for which the funds are needed, the anticipated cost of the project, amounts and sources of other funds available for the project, and the names of two or more scientific colleagues who are sufficiently familiar with the project to advise the AAAS Grants Committee regarding it.

For the purpose of administering these annual grants, the AAAS Grants Committee will be revived, and the names of the members will be published in a subsequent issue of SCIENCE.

All applications should be submitted *in triplicate* to Howard A. Meyerhoff, Administrative Secretary, American Association for the Advancement of Science, 1515 Massachusetts Ave., N.W., Washington 5, D. C.

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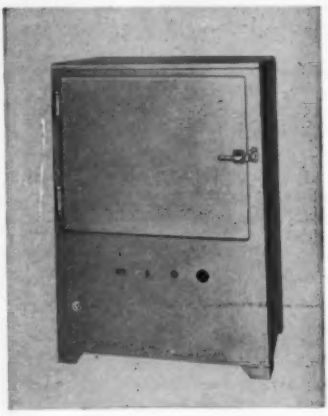

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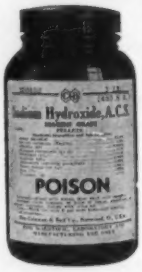

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Sept. 23–24. Institution of Mining and Metallurgy. Symposium on Mineral Dressing. London.

Sept. 23–26. American Roentgen Ray Society. Houston, Tex.

Sept. 23–Oct. 1. Congress of the International Scientific Film Association. La Maison de la Chimie, Paris.

Sept. 26–28. Aero Medical Association (Interim Meeting). Paris.

Sept. 27–28. Biological Photographic Association, Upper Midwest Chapter. University of Minnesota, Minneapolis.

Sept. 27–Oct. 1. Inter-American Congress of Public Health. Havana.

Sept. 27–Oct. 4. International Congress of Industrial Heating. Paris.

Sept. 29–Oct. 1. Association of Official Agricultural Chemists (Annual). Shoreham Hotel, Washington, D. C.

Sept. 29–Oct. 4. VIth Congrès International de Chauffage Industriel (Thermique et Thermodynamique appliquées). Paris.

Oct. 1–2. Association of Feed Control Officials. Shoreham Hotel, Washington, D. C.

Oct. 1–3. International Council of Scientific Unions (General Assembly). Amsterdam.

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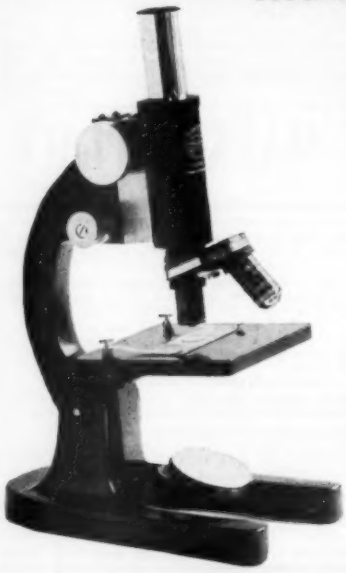

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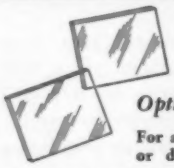

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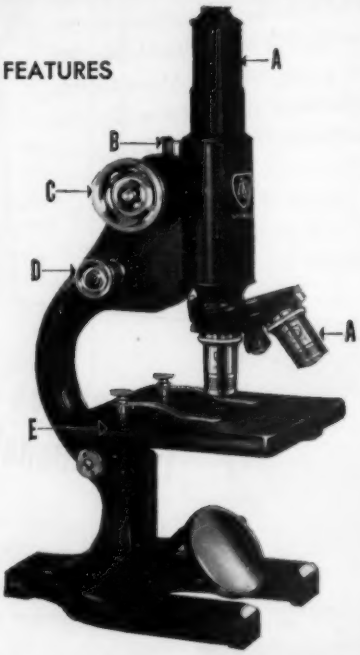

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